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# SOIL SCIENCE

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## CONTENTS

Influence of Soil Acidity upon the Decomposition of Organic Matter in Soils. J. W. WHITE, F. J. HOLBEN, AND C. D. JEFFRIES . . . . .	1
The Settling Volume of Soils. H. E. MIDDLETON AND H. G. BYERS . . . . .	15
An Examination of the Degtjareff Method for Determining Soil Organic Matter, and a Proposed Modification of the Chromic Acid Titration Method. A WALKLEY AND I ARMSTRONG BLACK . . . . .	29
A Critical Examination of Analytical Methods Used in the Determination of Exchangeable Potassium and Sodium of Soils. M. L. M. SALGADO . . . . .	39
An Electrometric-Titration Method of Finding the pH Value and Lime Requirement of Soils. AMAR NATH PURI AND BALMOKAND ANAND . . . . .	49
A New Apparatus for Measuring Soil Shrinkage AMAR NATH PURI, E. MCKENZIE TAYLOR, AND A. G. ASGHAR . . . . .	59
Oxidation Reduction Potentials in Soils. I. Principles and Electrometric Determination. LINDSEY A. BROWN . . . . .	65
Book Review . . . . .	77
The Influence of Copper Sulfate on Iron Absorption by Corn Plants L. G. WILLIS AND J R PILAND . . . . .	79
The Effect of Sulfur and Phosphorus on the Availability of Iron to Pineapple and Maize Plants. C. P. SIDERIS AND B H KRAUSS . . . . .	85
The Relation Between Replaceable Potassium and Field Response to Potash in Hawaiian Soils. O C MAGISTAD . . . . .	99
A New Method of Determining Base Exchange Capacity of Soils AMAR NATH PURI. 105	
Studies on Tropical Soil Microbiology: I The Evolution of Carbon Dioxide from the Soil and the Bacterial Growth Curve A STEVEN CORBET . . . . .	109
A Study of Rhizobium Species in Relation to Nodule Formation on the Roots of Florida Legumes. I W. R. CARROLL . . . . .	117
Studies on Podzols and Brown Forest Soils I KARL LUNDBLAD . . . . .	137
The Nature of Slick Soil in Southern Idaho. PHILIP ISAAC . . . . .	157
Influence of Eight Years' Successive Fertilizing with Concentrated Potash Salts and with Kainit on the Replaceable Bases of the "Terra Roxa" Soil THEODURETO DE CAMARGO AND P. C. DE MELLO . . . . .	167
The Relation of Colloid Dispersion in Soils to Chemical Changes Induced by Biological Transformations of Organic Materials. JOHN P. CONRAD . . . . .	179
The Influence of the pH of a Culture Solution on the Rates of Absorption of Ammonium and Nitrate Nitrogen by the Tomato Plant H E CLARK AND J W SHIVE . . . . .	203
A Study of Rhizobium Species in Relation to Nodule Formation on the Roots of Florida Legumes: II. W. R. CARROLL . . . . .	227
A Migration Method for the Determination of Replaceable Bases in Soils. LAWRENCE C. WHEETING . . . . .	243
Electrodialysis as a Means of Studying the Nature of Soil Phosphates L. A. DEAN . . . . .	253
The Fixation of Potash in Difficultly Available Form in Soils N J VOLK . . . . .	267
The Distinction between Magnesium Absorbed and That Exchangeable, Four Years after Lysimeter Incorporations of Oxides and Carbonates W. H. MACINTIRE, W. M. SHAW AND BROOKS ROBINSON . . . . .	289
Calcium Cyanamid as a Nitrogenous Fertilizer. T. R. MOYER . . . . .	305
Preservation of Soils Against Degeneration. W. L. POWERS . . . . .	333

Phosphate Fixation and Penetration in Soils. A. FLOYD HECK	343
The Influence of the Hydrogen-Ion Concentration of the Culture Solution upon the Absorption and Assimilation of Nitrate and Ammonium Nitrogen by Peach Trees Grown in Sand Cultures. O. W. DAVIDSON AND J. W. SHIVE	357
Physiological Studies on Rhizobium. I. The Effect of Nitrogen Source on Oxygen Consumption by Rhizobium Leguminosarum Frank. R. H. WALKER, D. A. ANDERSON AND P. E. BROWN	387
Laws of Soil Colloidal Behavior. XIV. Aging of Colloids and Base Exchange A. J. PUGH	403
Sven Odén, 1888-1934 H. LUNDEGÅRDH	429
The Cause of Decreased Nodule Formation on Legumes Supplied with Abundant Combined Nitrogen FRANKLIN E. ALLISON AND C. A. LUDWIG	431
Plant Feeding by Briquette Fertilization FRANCIS E. HANCE	457
The Influence of the pH of a Culture Solution on the Assimilation of Ammonium and Nitrate Nitrogen by the Tomato Plant H. E. CLARK AND J. W. SHIVE	476
A Method for Determining the Capacity of a Soil to Fix Phosphorus in Difficultly Available Form A. FLOYD HECK	482
A Comparison of Superphosphate and Raw Rock Phosphate on Different Types of Soil. A. W. BLAIR, A. L. PRINCE AND S. H. WINTERBERG	483

# ILLUSTRATIONS

## PLATES

### THE SETTLING VOLUME OF SOILS

Plate 1	Settling Volumes of A Horizons	27
---------	--------------------------------	----

### THE INFLUENCE OF COPPER SULFATE ON IRON ABSORPTION BY CORN PLANTS

Plate 1	Effect of Copper Sulfate on Iron Absorption by Corn Plants	83
Fig 1	Corn plants on peat soil with lime alone	83
2	Corn plants on peat soil with lime and copper sulfate	83
3	Iron accumulation in lower nodes of corn plants	83

### A STUDY OF RHIZOBIUM SPECIES IN RELATION TO NODULE FORMATION ON THE ROOTS OF FLORIDA LEGUMES. I

Plate 1	Fig 1	Soybean Culture 6, a Slowly Growing, Inefficient Nitrogen Fixer	135
	2	Soybean Culture 68, a More Rapidly Growing, Highly Efficient Nitrogen-Fixing Rhizobium	135
	3	Dimensions of the Larger Nodules of <i>Wistaria chinensis</i>	135
	4	A Dense Growth of <i>Crotalaria spectabilis</i> , One of the Hardier Species	135

### A STUDY OF RHIZOBIUM SPECIES IN RELATION TO NODULE FORMATION ON THE ROOTS OF FLORIDA LEGUMES. II

Plate 1	Effect of Inoculation with Various Soybean and Cowpea Cultures on the Growth of Soybean and Cowpea Plants	239
	Fig 1 Efficiency of soybean and cowpea nodule bacteria in symbiosis with the cowpea plant	239
	2 Soybean plants showing variation in growth due to differences in nitrogen-fixing power of various cultures of soybean and cowpea nodule bacteria	239
	3. Variation in growth due to the effect of different cultures on the cowpea plant	239
Plate 2	Fig 1 The well-inoculated roots of cowpea plants in small plat 5 shown in plate 1, fig 3	241
	2. Morphological forms of cowpea nodule bacteria, culture 600 grown in asparagus-mannitol solution	241

### CALCIUM CYANAMID AS A NITROGENOUS FERTILIZER

Plate 1	Fig 1.	Sixty frames constructed from 2 inch by 12 inch boards	331
	2.	Settling of soil colloids	331

### THE INFLUENCE OF THE HYDROGEN-ION CONCENTRATION OF THE CULTURE SOLUTION UPON THE ABSORPTION AND ASSIMILATION OF NITRATE AND AMMONIUM NITROGEN BY PEACH TREES GROWN IN SAND CULTURES

Plate 1.	Fig. 1.	Representative trees grown in the ammonium series	383
	2.	Representative trees grown in the nitrate series	383
Plate 2.	Fig. 1.	Roots of representative trees grown in the ammonium series	385
	2.	Roots of representative trees grown in the nitrate series	385

PHYSIOLOGICAL STUDIES ON RHIZOBIUM: I. THE EFFECT OF NITROGEN SOURCE ON OXYGEN CONSUMPTION BY RHIZOBIUM LEGUMINOSARUM FRANK

Plate 1. Apparatus for Measuring the Effects of Nitrogen on Rhizobium . . . . .	401
Fig. 1. Respiration vessel. . . . .	401
2. Simple manometer of the Warburg type with respiration vessel attached. . . . .	401
3. Water bath in which the temperature of the water surrounding the respiration vessels was thermostatically controlled within 0.01°C . . . . .	401

LAWS OF SOIL COLLOIDAL BEHAVIOR: XIV. AGING OF COLLOIDS AND BASE EXCHANGE

Plate 1. Effect of Aging on the Stability of Aluminum Silicate in a Solution of Barium Acetate . . . . .	427
--	-----

PLANT FEEDING BY BRIQUETTE FERTILIZATION

Plate 1. Effect of Briquette Fertilization on Papaya and Eucalyptus Seedlings . . . . .	453
Fig 1. Indicator plant: Papaya seedlings . . . . .	453
2. Indicator plant: <i>Eucalyptus ovata</i> . . . . .	453
Plate 2. Fig. 1. Cement type of slowly disintegrating fertilizer briquette . . . . .	455
2 Indicator plants: Papaya seedlings and <i>Eucalyptus ovata</i> . . . . .	455
Plate 3. Fig 1 Briquette fertilization of sugar cane . . . . .	457
2 Types of briquettes . . . . .	457

TEXT-FIGURES

INFLUENCE OF SOIL ACIDITY UPON THE DECOMPOSITION OF ORGANIC MATTER IN SOILS

Fig 1. Course of Carbon Dioxide Production in 14 Days from the Six Soils as the Results of Different Treatments . . . . .	8
2. Relative Carbon Dioxide Producing Capacity of the Six Soils as Influenced by various Treatments . . . . .	9

THE SETTLING VOLUME OF SOILS

Fig 1 Settling Volumes of A Horizons . . . . .	18
2 Settling Volumes of B Horizons . . . . .	18
3. Relation of Settling Volume to Moisture Equivalent . . . . .	21
4 Relation of Settling Volume of Colloids to Silica-Sesquioxide Ratio . . . . .	23
5 Water Relationships of Erosion Station Surface Soils . . . . .	24

AN EXAMINATION OF THE DEGTJAREFF METHOD FOR DETERMINING SOIL ORGANIC MATTER, AND A PROPOSED MODIFICATION OF THE CHROMIC ACID TITRATION METHOD

Fig. 1 Relations Between Total Carbon as Obtained by Dry Combustion and Carbon as Obtained by the Reduction Methods Using Chromic Acid and Either Hydrogen Peroxide or Water . . . . .	33
--	----

AN ELECTROMETRIC-TITRATION METHOD OF FINDING THE pH VALUE AND LIME REQUIREMENT OF SOILS

Fig. 1. Arrangement of Apparatus in the Electrometric-Titration Method . . . . .	50
2 Titration Curve of the Universal Buffer Solution . . . . .	51

A NEW APPARATUS FOR MEASURING SOIL SHRINKAGE

Fig 1 Diagram of Shrinkage Measuring Apparatus . . . . .	60
2 Typical Shrinkage Curves of Soils . . . . .	62

# OXIDATION REDUCTION POTENTIALS IN SOILS. I. PRINCIPLES AND ELECTROMETRIC DETERMINATION

Fig. 1. Type of Half Cell Used. . . . .	69
---	----

## THE EFFECT OF SULFUR AND PHOSPHORUS ON THE AVAILABILITY OF IRON TO PINEAPPLE AND MAIZE PLANTS

Fig. 1. Average Annual Rainfall, Total Replaceable Bases, and pH of Soils on the Slope between Lower Brodie and Upper Helemano Districts on the Island of Oahm. . . . .	86
2. Water-soluble Iron Concentrations at Different pH Values of Certain Soils of Pineapple Fields . . . . .	88

## THE RELATION BETWEEN REPLACEABLE POTASSIUM AND FIELD RESPONSE TO POTASH IN HAWAIIAN SOILS

Fig. 1. Harvest Yields of Pineapple Fruits in Field Experiments with Varying Potash Fertilization . . . . .	101
---	-----

## STUDIES ON TROPICAL SOIL MICROBIOLOGY. I. THE EVOLUTION OF CARBON DIOXIDE FROM THE SOIL AND THE BACTERIAL GROWTH CURVE

Fig 1 . . . . .	112
-----------------	-----

## STUDIES ON PODZOLS AND BROWN FOREST SOILS. I

Fig 1 The Loss on Ignition and the Amounts of Silica and Sesquioxides Extracted with the Acid-Oxalate Method, Per Cent of Oven-Dry Soil, in Profile 1 . . . . .	150
2 The Loss on Ignition and the Amounts of Silica and Sesquioxides Extracted with the Acid-Oxalate Method, Per Cent of Oven-Dry Soil, in Profile 3. . . . .	150
3 The Loss on Ignition and the Amounts of Silica and Sesquioxides Extracted with the Acid-Oxalate Method, Per Cent of Oven-Dry Soil, in Profile 4 . . . . .	152
4 The Loss on Ignition and the Amounts of Silica and Sesquioxides Extracted with the Acid-Oxalate Method, Per Cent of Oven-Dry Soil, in Profile 5 . . . . .	152
5. The Loss on Ignition and the Amounts of Silica and Sesquioxides Extracted with the Acid-Oxalate Method, Per Cent of Oven-Dry Soil, in Profile 7 . . . . .	153

## INFLUENCE OF EIGHT YEARS' SUCCESSIVE FERTILIZING WITH CONCENTRATED POTASH SALTS AND WITH KAINIT ON THE REPLACEABLE BASES OF THE "TERRA ROXA" SOIL

Fig 1 Graphs of Buffer Surface and Buffer Numbers of Soil Samples Treated with Different Quantities of Tenth-Normal Hydrochloric Acid . . . . .	172
2 The pH Values of Soil Samples Treated with Different Quantities of Tenth-Normal Calcium Hydroxide. . . . .	172

## THE INFLUENCE OF THE pH OF A CULTURE SOLUTION ON THE RATES OF ABSORPTION OF AMMONIUM AND NITRATE NITROGEN BY THE TOMATO PLANT

Fig 1. Milligrams of Nitrogen Absorbed per 100 Gm Green Tissue in 6 Hours by Plants 41 Days Old . . . . .	210
2. Milligrams of Nitrogen Absorbed per 100 Gm Green Tissue in 6 Hours by Plants 52 Days Old . . . . .	213
3. Milligrams of Nitrogen Absorbed per Gram Dry Plant Tissue in 6 Hours by Plants 38 Days Old . . . . .	216

## A MIGRATION METHOD FOR THE DETERMINATION OF REPLACEABLE BASES IN SOILS

Fig. 1. Experimental Arrangement for Extracting the Bases from Soils by the Migration Method. . . . .	246
---	-----



## ELECTRODIALYSIS AS A MEANS OF STUDYING THE NATURE OF SOIL PHOSPHATES

Fig. 1. Rates of Removal by Electrodialysis of Phosphorus from Tribasic Calcium Phosphates and Rock Phosphate.....	255
2. Rates of Removal by Electrodialysis of Phosphorus from Soil Untreated and Treated with Calcium Phosphates.....	255
3. Rates of Removal by Electrodialysis of Phosphorus from Difficultly Soluble Phosphate Compounds.....	257
4. Rates of Removal by Electrodialysis of Phosphorus from Soils Containing Difficultly Soluble Phosphate Compounds.....	257
5. A Comparison of the Rates of Removal of Phosphorus by Electrodialysis from Hawaiian Soil 4391 and from Iron and Aluminum Phosphates, All Samples Containing Equal Quantities of Phosphorus.....	260
6. Rate of Removal of Phosphorus by Electrodialysis from a Treated and an Untreated Soil and from Dufrenite. Also a Curve Based on the Sum of the Corresponding Ordinates of the Two Lower Curves.....	264

## THE FIXATION OF POTASH IN DIFFICULTLY AVAILABLE FORM IN SOILS

Fig. 1. Effect of the Concentration of Soluble Potash on the Amount Fixed by Miami Silt Loam When Treated with Varying Quantities of Potassium Bicarbonate and Then Alternately Wetted and Dried 10 Times at 70°C.....	270
2. Potash Content of the Textural Separates of Four Soils.....	271
3. Amounts of Potash per Acre Fixed in Difficultly Available Form by the Textural Separates of Four Soils Treated With 60 Tons per Acre of Potash and Alternately Wetted and Dried 10 Times at 70°C.....	272
4. A Comparison of the X-Ray Pattern of Pure Muscovite with the Patterns of the Heavy Colloidal Fractions of the Potassium Chloride and Check Plats of Hagerstown Silt Loam, Showing Muscovite to be More Plentiful in the Potassium Chloride Plat.....	282

## THE DISTINCTION BETWEEN MAGNESIUM ABSORBED AND THAT EXCHANGEABLE FOUR YEARS AFTER LYSIMETER INCORPORATIONS OF OXIDES AND CARBONATES

Fig. 1. Comparison of Four Procedures in Effecting Recoveries of the Magnesium Residues from 32-Ton CaO $\rightleftharpoons$ Additions of Oxide, Hydroxy Carbonate, and Normal Carbonate 4 Years after Incorporations in Outdoor Lysimeters.....	300
--	-----

## PHOSPHATE FIXATION AND PENETRATION IN SOILS

Fig. 1. The Relation of Calcium and Active Aluminum and Iron to the Form and Availability of Applied Phosphorus Fixed in the Soil.....	344
2. Rate of Solution or Solubility Curves for Known Phosphates and for the Native and Applied Phosphates in Soils.....	346
3. The Relative Amounts of Availability of the Phosphates Formed in Different Soils From Applied Soluble Phosphate.....	348
4. The Downward Penetration of Phosphorus and Its Recovery in Readily Available Form.....	350

## THE INFLUENCE OF THE HYDROGEN-ION CONCENTRATION OF THE CULTURE SOLUTION UPON THE ABSORPTION AND ASSIMILATION OF NITRATE AND AMMONIUM NITROGEN BY PEACH TREES GROWN IN SAND CULTURES

Fig. 1. Average Dry Weight Yields in Grams and Average Absorption Rates in Milligrams per Gram of Dry Plant Material per Hour.....	368
2. Average Growth Increments as Indicated by Increase in Height of Main Stems per Day.....	369

3. Inorganic Nitrogen in Peach Roots Expressed in Per Cent of Dry Matter. Rates of Absorption of Inorganic Nitrogen by Peach Trees Expressed in Milligrams per Gram of Dry Matter per Hour..... 375
4. Nitrogenous Fractions in Stems of Peach Trees Expressed in Per Cent Green Matter, and in Per Cent Total Organic Nitrogen..... 376
5. Nitrogenous Fractions in Peach Roots Expressed in Per Cent Green Matter, and in Per Cent Total Organic Nitrogen..... 377

PHYSIOLOGICAL STUDIES ON RHIZOBIUM: I. THE EFFECT OF NITROGEN SOURCE ON OXYGEN CONSUMPTION BY RHIZOBIUM LEGUMINOSARUM FRANK

- Fig. 1. The Rate of Oxygen Consumption by *Rhizobium leguminosarum* in Yeast Extract Media..... 392
2. The Rate of Oxygen Consumption by *Rhizobium leguminosarum* in Media Containing Ammonium Chloride, Sodium Nitrate, Urea, and Alanine..... 395
  3. Ratio of the Amount of Oxygen Consumed in 30 Hours in Media Containing Various Nitrogen Sources to that Consumed in a Nitrogen-Free Medium..... 395

THE INFLUENCE OF THE pH OF A CULTURE SOLUTION ON THE ASSIMILATION OF AMMONIUM AND NITRATE NITROGEN BY THE TOMATO PLANT

- Fig. 1. Nitrate Nitrogen in Tomato Roots and Rates of Absorption Nitrate Nitrogen from Culture Solutions..... 465
2. Ammonia Nitrogen in Tomato Roots and Rates of Absorption of Ammonia Nitrogen from Culture Solutions..... 466
  3. Ammonia Nitrogen, Basic-Free Amino Nitrogen, and Soluble Organic Nitrogen in Tomato Roots..... 467
  4. Total Soluble Organic Nitrogen in Leaves, Stems, and Roots of Tomato Plants. 473

A METHOD FOR DETERMINING THE CAPACITY OF A SOIL TO FIX PHOSPHORUS IN DIFFICULTLY AVAILABLE FORM

- Fig. 1. The Recovery of Applied Phosphorus Fixed in Moist Soils in Readily Available Form, and the Relation of the Direct and Corrected Recovery Values of the Proposed Method of These Data..... 478



# INFLUENCE OF SOIL ACIDITY UPON THE DECOMPOSITION OF ORGANIC MATTER IN SOILS<sup>1</sup>

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Soil investigations concerning factors which influence the rate of decay of organic materials have been confined primarily to studies of the stimulating effects of various soluble salts with special reference to available nitrogen and phosphorus.

Waksman and Heukelekian (3) state that there is no correlation between soil reaction and its cellulose decomposing capacity. Their conclusions are followed by the statement that fungi which take a very active part in the decomposition of cellulose in the soil will thrive readily even under the most acid conditions in which cultivated plants will barely grow at all. The authors state that similar results were obtained by Christensen, Carpenter, and Barthel and Bengtsson. In a second article dealing with carbon dioxide evolution, Waksman and Starkey (4) conclude that the addition of lime to an acid soil makes conditions more favorable for the activities of microorganisms, thus resulting in an increase in the number of bacteria (decrease of fungi) and an increase in the respiratory power of the soil.

Stoklasa and Doerell (2) found that 1 kgm. of soil of pH 7.2 produced in 24 hours 85 mgm. of carbon dioxide compared with 5 mgm. produced by a soil of pH 4.0. In their discussion of these and similar data, the authors point out the fact that soils which vary between pH 4.7 and 4.0 are frequently noticeably rich in decomposable organic substances but that the acidity is so great that the bacteria can not properly develop.

It is a well-known fact that the rate of decay of organic materials in the soil is dependent upon the nature, number, and activity of soil microorganisms. The decomposition of both the residual organic matter (respiratory capacity) and applied organic materials (organic matter decomposing power) should be affected, therefore, by any soil addition or treatment that may influence the biological properties of the soil. The studies of Waksman and Heukelekian (3) on certain limed and unlimed field plot soils, show that the addition of available nitrogen, which had little influence on soil reaction, increased the unlimed soils to a cellulose decomposing capacity comparable with that of the limed soils. There are, however, no published data dealing with such studies based on a wide range in soil reaction in which the studies were undertaken primarily to determine the influence of soil acidity upon the organic matter decomposing power of soils.

## METHODS

The terms "respiratory capacity" and "organic matter decomposing power of soils" as used in this text are in accordance with the definitions proposed by Waksman and Starkey (4). The former term relates to the decay of residual soil organic matter, and the latter, to the decay of applied organic materials.

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<sup>1</sup> Publication authorized by the director of the Pennsylvania Agricultural Experiment Station May 18, 1933 as Technical Paper No. 591. Contribution from the department of agronomy.

<sup>2</sup> Professor and assistant professors of soil technology respectively.

The influence of soil acidity upon the decomposition of organic matter is expressed on the basis of 250 gm. of dry soil. The soils of different pH values were treated with the several organic materials followed by a 2-week incubation period at room temperature. Water was added in amounts equivalent to 55 per cent of the water-holding capacity. The soils, placed in a series of 1-liter side-neck filter flasks, were kept in a dark cupboard during the incubation period. At 2-day intervals the accumulated carbon dioxide was measured by absorption in a train of soda-lime tubes arranged in the usual way. Seven flasks, arranged in series, were aspirated for 40 minutes, during which time 4 liters of CO<sub>2</sub>-free air were drawn through the apparatus. The respiratory capacity of the untreated soils and the organic matter decomposing power of the treated soils were thus measured in terms of the weight of carbon dioxide produced by each 250 gm. of soil.

#### ORGANIC MATERIALS USED

Four organic materials were included in the study; namely, manure, cellulose (ground filter paper), cotton seed meal, and corn starch. The materials were applied at the rate of 0.5 per cent with the exception of manure, which was used at the rate of 1.0 per cent. The manure obtained from the Institute of Animal Nutrition, represented a sample of steer dung free from urine and litter.

The nitrogen as nitrate of soda was applied at a uniform rate equivalent to 42 mgm. of nitrogen. Dipotassium phosphate (K<sub>2</sub>HPO<sub>4</sub>) was applied at the rate of 50 mgm. Hydrated lime was applied as follows: soil Nos. 1 and 2 received no lime; No. 3, 0.172 per cent; No. 4, 0.272 per cent; No. 5, 0.319 per cent; and No. 6, 0.360 per cent.

The lime was applied to the air-dried acid soils, which were made up to optimum moisture content and incubated for 1 month. The alkaline and neutral soils, numbers 1 and 2, were incubated for the same period but received no lime. At the end of 1 month, the six soils were air-dried and treated with cellulose and nitrogen as indicated.

#### SOIL

The use of field soils of unknown history for such studies, selected on the basis of differences in pH range does not represent a true measure of the influence of soil reaction on the rate of decay of organic materials. As the result of differences in previous field treatments, such soils may differ widely in composition and properties other than that of soil acidity. In order, therefore, to obviate these possible variables, soils were used which had been previously prepared in the following manner. A stock soil made up of 5 parts by weight of Hagerstown silt loam from a permanent sod, 1 part air-dried sewage sludge, and 1 part of fine sand was divided into 10 equal parts of 260 pounds. Nine portions of this stock soil were treated with different amounts of sulfur. The nine sulfur-treated soils and also one portion untreated with sulfur, were arranged in a series of small field plots, 3 feet by 1.5 feet by 1 foot deep. Each

of the 10 plots were bounded by boards of appropriate width. At the end of 2 years, during which time the plots were left undisturbed (except for the removal of occasional small samples), representative samples were taken from each of the 10 plots, air dried, and passed through a 6-mesh screen. From these 10 samples, 6 which showed a maximum pH range between 7.4 and 3.2 were selected for study.

The six soils thus prepared, including also a sample of the original stock soil, were analyzed to include the following determinations—pH, total nitrogen, nitric nitrogen, organic carbon, available phosphorus (Truog Method), water-soluble inorganic salts, and water-soluble organic matter. The relative nitrifying capacity of the six plot soils and also the number of microorganisms per gram of soil were determined. The sewage sludge used contained

TABLE 1  
*Nature of soils used in respiration studies*

SOIL NUMBER	REACTION	SULFUR APPLIED (1930)	TOTAL N	ORGANIC C	NITRIC N	AVAILA- BLE P†	WATER- SOLUBLE INORGANIC SALTS‡	WATER- SOLUBLE ORGANIC MATTER
	pH		per cent			mgm. per 250 gm. soil		
Stock soil*.	6.3	.....	0.322	3.45	1 0	26.0	192.5	257.5
1	7.4	.....	0.171	2.08	1.5	18.5	95.0	25.0
2	7.1	0.025	0.162	1.96	1.8	23.0	93.8	22.5
3	6.0	0.075	0.165	1.98	1.0	12.8	121.2	43.8
4	4.5	0.200	0.165	2.01	1.8	13.3	418.7	41.2
5	3.8	0.350	0.159	1.85	1.8	9.0	583.8	36.2
6	3.2	0.600	0.164	1.95	3.5	7.5	822.5	56.3

\* Stock soil made up of 5 parts Hagerstown silt loam, 1 part sewage sludge and 1 part sand (by weight).

† Truog method.

‡ The excess of inorganic salts in soils 3 to 6 consists largely of calcium sulfate.

the equivalent of 3.47 per cent calcium carbonate, 3.4 per cent nitrogen, 1.28 per cent phosphoric acid ( $P_2O_5$ ), 17.3 per cent organic carbon, and an appreciable amount of water-soluble potash.

#### EFFECT OF SULFUR ON THE COMPOSITION OF THE SIX PLOT SOILS

Table 1 shows the amounts of sulfur applied to the field plots and the composition of the soils at the end of 2 years. During the 2-year period the soils lost, on an average, 49 per cent of the original nitrogen and 42 per cent of the carbon. As a result of these proportionate losses, the nitrogen-carbon ratio changed from 10.7 to approximately that of the original Hagerstown soil (12.8). During this period of organic matter decomposition the basic ash constituents of the compost were deposited in the soils, which accounts for the increased alkalinity of the first two soils of the series. There were two major factors in operation during the process of organic matter decay, each tending to

effect a change in soil reaction—the deposition of basic ash constituents of the compost and the oxidation of sulfur. The final reactions as measured by pH are shown in table 1. The abnormal acidity of soil number 6 is due to the presence of small amounts of free mineral acid. After the soil was leached with distilled water the pH approached that of soil number 5. The excess of water-soluble inorganic salts, and water-soluble inorganic salts present in the more acid soils is due to an accumulation of calcium sulfate made possible by reduced rainfall preceding the removal of the soil samples. The organic matter content of the six soils as measured by the total nitrogen and organic carbon remained fairly constant. The water-soluble organic matter, however, shows a greater accumulation in case of the more acid soils.

It becomes obvious that the changes in the nature of the six soils as determined at the end of 2 years may be attributed directly to the influence of soil acidity induced by the oxidation of the applied sulfur. These changes, as will be shown later, greatly influenced the biological properties of the several soils to which sulfur was applied. The wide differences to be noted, therefore, in the respiratory capacity and organic matter decomposing power of the six soils may be attributed to the controlling influence of soil acidity on the nature, number, and activities of the soil microorganisms.

In the interpretation of the experimental data presented in the succeeding tables, it is recognized that carbon dioxide is only one of several by-products of organic matter decay and that the fungi assimilate carbon derived from the decomposition of organic residues in a different proportion from that of bacteria. However, the assimilated carbon utilized in the synthesis of the microbial cells is eventually attacked by a new generation of microorganisms, resulting in the liberation of the carbon as carbon dioxide. There is available, in any event, sufficient experimental evidence to warrant the measurement of organic matter decay in terms of soil respiration or carbon dioxide evolution.

#### EXPERIMENTAL ERROR OF METHOD USED IN THE DETERMINATION OF THE RESPIRATORY CAPACITY AND ORGANIC MATTER DECOMPOSING POWER OF THE SIX SOILS OF DIFFERENT pH

Statistical studies were made on the variations in duplicate determinations of carbon dioxide recovered from similarly treated soils. Based on the total carbon dioxide recovered in 14 days involving 54 determinations in duplicate, the probable error of a single determination was found to be  $\pm 1.95$  per cent. A study of 378 duplicate determinations based on the carbon dioxide recovered at 2-day intervals gave a probable error of a single determination of  $\pm 5.2$  per cent.

The probable error involved in the procedure used as measured in terms of carbon dioxide recovered, includes, no doubt, the accumulative errors involved in the removal and weighing of duplicate samples, mixing of materials, addition of water, and manner of transferring wet soil to flasks as well as the absorption and weighing of the carbon dioxide. The higher error based on the 2-day

intervals may be expected so far as the errors due to the measurements of carbon dioxide are concerned, since the total carbon dioxide produced in 14 days represents the accumulative plus and minus differences for each 2-day period. The probable errors, however, are small as compared to the wide differences in carbon dioxide production of the six soils. The probable error data will serve as a means of determining the significance of the difference in behavior of the six soils with respect to the evolution of carbon dioxide.

### RESULTS

The total carbon dioxide produced by the six soils of different pH values in relation to the various treatments is shown in table 2. These data serve to emphasize the fact that soil reaction, under the condition of this experiment, plays an important rôle in the control of the biological activities of the six soils. This is especially true in regard to the respiratory capacity of the soils. The

TABLE 2  
*Production of carbon dioxide in relation to the various soil treatments*

SOIL NUMBER	REACTION OF SOILS	EVOLUTION OF CO <sub>2</sub> IN 14 DAYS									
		Soil alone	Soil + N	Soil + cellu- lose	Soil + cellu- lose + N	Soil + cellu- lose + N + K <sub>2</sub> HPO <sub>4</sub>	Soil + manure	Soil + manure + N	Soil + cotton- seed meal	Soil + cotton- seed meal + N	Soil + starch
	pH	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	7.4	335	411	957	1,219	1,196	856	879	1,326	1,429	1,148
2	7.1	269	324	873	1,144	1,160	857	807	1,223	1,364	1,039
3	6.0	262	370	790	1,171	1,151	774	801	1,141	1,315	1,138
4	4.5	191	322	625	797	747	555	694	1,137	1,230	1,140
5	3.8	105	164	514	733	745	353	436	954	952	1,058
6	3.2	112	155	450	488	550	267	309	888	940	1,043

two most acid soils show a value about one third that of the soil of pH 7.4. With the one exception of the starch treatment the production of carbon dioxide of the soils below pH 6.0 is considerably reduced. Apparently the most acid soils have a capacity for the decomposition of starch equal to that of the alkaline soils. The addition of soluble nitrogen failed to restore the acid soils to normal carbon dioxide production. Nitrogen stimulated the decay of the organic materials in each soil in about equal proportions and thus failed to change the relationship with respect to the relative carbon dioxide production of each soil. The addition of dipotassium phosphate to the cellulose-nitrogen treatment failed to increase the production of carbon dioxide except in case of the two most acid soils. This slight difference in the effect of the treatment caused little change in the relationship of the six soils.

The organic matter decomposing power of the six soils is shown in tables 3 and 4. The data included in these two tables are computed from the total milligrams of carbon dioxide included in table 2. The cellulose and manure



data are quite similar. In each case the decomposing power of the two alkaline soils is considerably greater than that of the acid soils. The power of the alkaline soils to decompose cotton seed meal is also much greater. The acid soils however have a greater power to bring about the decomposition of starch. The addition of soluble nitrogen had little effect in increasing the relative

TABLE 3  
*Organic matter decomposing power of the six soils*

SOIL NUMBER	REACTION OF SOILS	*EVOLUTION OF CO <sub>2</sub> IN 14 DAYS						
		No nitrogen added				Addition of 42 mgm. soluble nitrogen		
		Cellulose	Manure	Cotton-seed meal	Corn starch	Cellulose	Manure	Cotton-seed meal
	pH	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	7.4	622	521	991	813	808	468	1,018
2	7.1	604	588	954	770	820	483	1,040
3	6.0	528	512	879	876	801	431	945
4	4.5	434	364	946	949	475	372	908
5	3.8	399	248	849	953	569	272	788
6	3.2	338	155	776	931	333	154	785

\* Excess of that produced by soils without organic additions.

TABLE 4  
*Relative respiratory capacity and organic matter decomposing power of the six soils*

SOIL NUMBER	REACTION OF SOILS	* BASED ON EVOLUTION OF CO <sub>2</sub> IN 14 DAYS							
		Results expressed in terms of soil No. 1 taken as 100							
		No nitrogen added					Addition of 42 mgm. soluble nitrogen		
	pH	Soil	Cellulose	Manure	Cotton-seed meal	Corn starch	Soil	Cellulose	Cotton-seed meal
1	7.4	100	100	100	100	100	100	100	100
2	7.1	80	97	113	96	95	79	101	103
3	6.0	78	85	98	89	108	90	99	90
4	4.5	57	70	70	95	117	78	59	79
5	3.8	31	64	48	86	117	40	70	58
6	3.2	33	54	30	78	114	38	41	33

organic matter decomposing power of the acid soils. The data included in table 4 in which the results are expressed in terms of soil number 1 taken as 100 (percentage relations) show conclusively that the respiratory capacity and organic matter decomposing power of the six soils (excluding the starch treatment) are parallel to the reaction of the soils. In terms of the probable error, the soils of pH below 6.0 show a significant decrease in both the respiratory capacity and the organic matter decomposing power as compared with the

soils of pH 7.4 and 7.1. The results are so conclusive that it seems unnecessary to discuss further these data.

### *Effect of lime*

In order to determine the effect of lime as a means of restoring the acid soils to normal biological activity, the four acid soils were treated with lime in amounts shown in table 5. The four limed soils together with those not limed were made up to optimum moisture content and incubated for 30 days. The soils were then air dried, treated with cellulose and cellulose-nitrogen and incubated for 2 weeks, during which time the carbon dioxide was recovered at 2-day periods in the manner already described.

The soils were treated with lime prior to the 14-day incubation in order to allow time for the soils to reach a state of equilibrium following the chemical and biological changes induced by the effect of lime. The data in table 5 show

TABLE 5  
*Influence of lime upon the carbon dioxide production of the four acid soils treated with  
cellulose*

SOIL NUMBER	Ca(OH) <sub>2</sub> APPLIED*	REACTIONS OF SOILS		EVOLUTION OF CO <sub>2</sub> IN 14 DAYS	
		Before liming	After liming	Soil + cellulose + Ca(OH) <sub>2</sub>	Soil + cellulose + Ca(OH) <sub>2</sub> + N
	<i>per cent</i>	<i>pH</i>	<i>pH</i>	<i>mgm.</i>	<i>mgm.</i>
1	.....	7.4	7.0	804	669
2	.....	7.1	6.6	660	717
3	0.172	6.0	7.2	692	724
4	0.272	4.5	6.8	709	710
5	0.319	3.8	6.7	749	748
6	0.360	3.2	6.3	821	712

\* Soils 1 and 2 received no lime.

that the lime and nitrogen treatment restored the acid soils to normal biological activity. The failure of nitrogen to cause an increased production of carbon dioxide in case of several of the soils may be due to the fact that during the 30-day incubation period sufficient nitrates were formed to meet the soils' need. Unfortunately insufficient lime was applied to bring the three most acid soils to the same pH as that of soil number 1. The data seem to show that lime is the major limiting factor and that nitrogen is also of importance as a controlling factor in the acid soils. Figure 1 shows the relative production of carbon dioxide from the limed and unlimed cellulose treated soils.

### *Influence of length of incubation period on the relationship of the six soils with respect to carbon dioxide production*

The results included in tables 2, 3, 4, and 5 relate to the total carbon dioxide produced at three periods of incubation in relation to several different treatments and shows that the relationship of the six soils with respect to the three

periods is approximately the same. These data therefore point to the fact that a short period of incubation as a means of establishing the relative respiratory

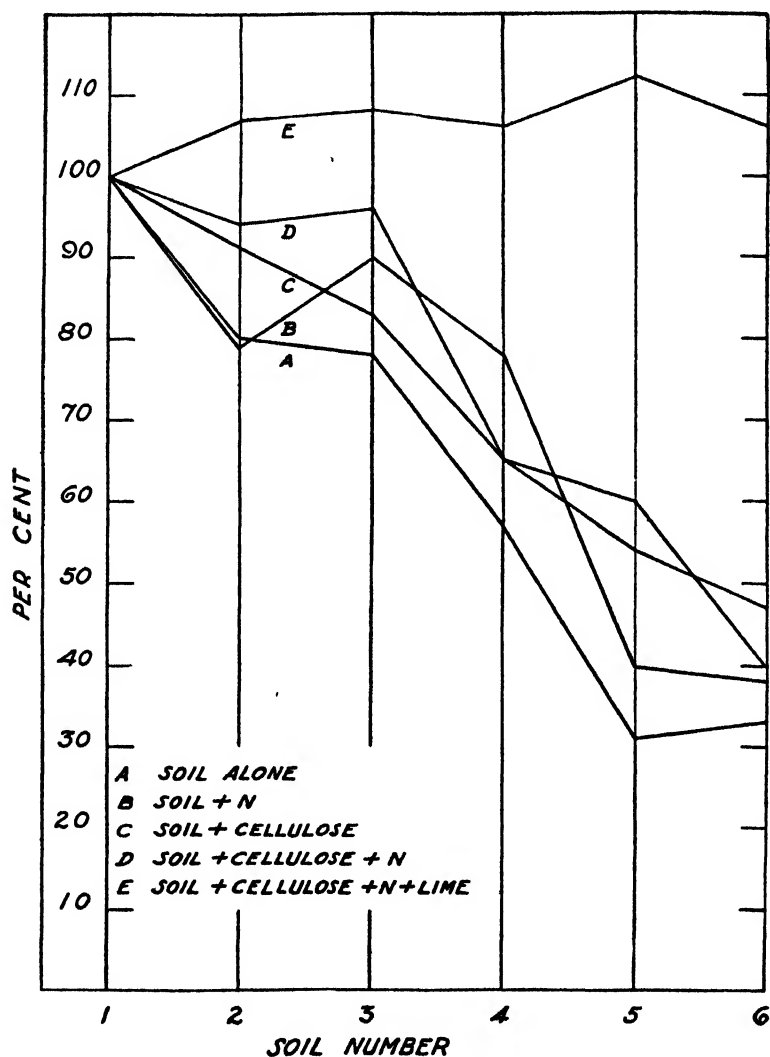


FIG. 1. COURSE OF CARBON DIOXIDE PRODUCTION IN 14 DAYS FROM THE SIX SOILS AS THE RESULTS OF DIFFERENT TREATMENTS

Data computed in terms of soil number 1 of each series taken as 100. The effect of lime in restoring the biological activities of the acid soils (numbers 3, 4, 5, 6) is shown from a comparison of series D and E.

capacity and organic matter decomposing power of several soils is comparable with that of a longer period. It is believed, however, that to measure the effect of a treatment such as lime, which brings about pronounced chemical and

biological soil changes, can not be accurately determined within a short period of incubation. The value of lime in restoring the biological activity of the four

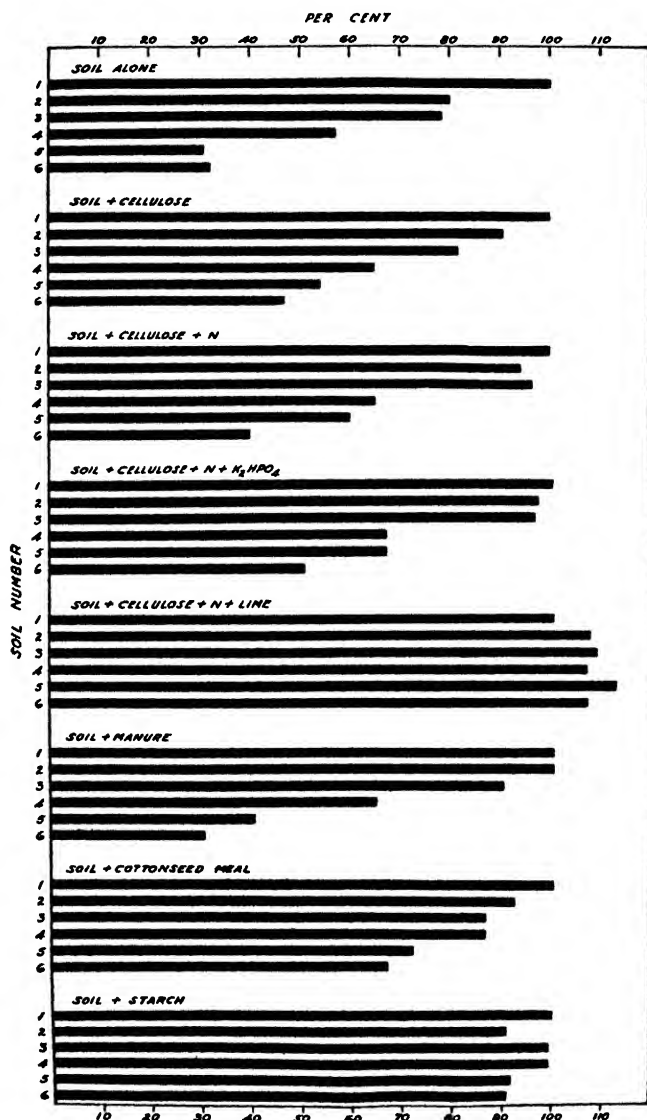


FIG. 2. RELATIVE CARBON DIOXIDE PRODUCING CAPACITY OF THE SIX SOILS AS INFLUENCED BY VARIOUS TREATMENTS

Results based on the total carbon dioxide produced in 14 days in terms of soil number 1 of each series taken as 100.

acid soils is clearly shown by comparison of the data involving the soil + cellulose + nitrogen treatment with the same treatment including the addition

of hydrated lime. Figure 2 shows the relative production of carbon dioxide in relation to eight representative soil treatments.

*Respiratory capacity and cellulose decomposing power of the six soils based on the fresh screened soil not air dried*

It is a well-known fact that the process of air drying soils stimulates the activities of microorganisms as the result of an increased supply of soluble salts and

TABLE 6

*Influence of length of incubation period on the relative production of carbon dioxide by the six soils*

SOIL NUMBER	RESULTS EXPRESSED IN TERMS OF SOIL NO. 1 TAKEN AS 100														
	Based on the total CO <sub>2</sub> produced in 2, 8, and 14 days														
	Soil alone			Soil + N			Soil + cellulose			Soil + cellulose + N			Soil + cellulose + N + Ca(OH) <sub>2</sub>		
	Days			Days			Days			Days			Days		
	2	8	14	2	8	14	2	8	14	2	8	14	2	8	14
1	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
2	79	79	80	77	77	79	90	91	91	82	88	94	102	103	107
3	78	78	78	85	91	90	80	81	83	87	93	96	98	93	108
4	58	57	57	63	75	78	63	66	65	58	63	65	104	99	106
5	30	30	31	27	36	40	31	48	54	30	55	60	100	119	112
6	24	29	33	26	33	38	27	33	47	26	34	40	120	113	106

TABLE 7

*Respiratory capacity and cellulose decomposing power of the fresh screened soil—not air dried\**

SOIL NUMBER	REACTION OF SOILS	EVOLUTION OF CO <sub>2</sub> IN 14 DAYS					
		In soil + cellulose	In soil alone	Due to the addition of cellulose	In soil + N + cellulose	In soil + N	Due to the addition of cellulose
	pH	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	7.3	533	213	320	879	107	772
2	7.0	427	168	259	797	98	699
3	6.4	356	162	194	804	95	709
4	5.2	272	113	159	669	97	572
5	4.4	325	72	253	378	70	308
6	3.9	198	74	124	341	83	258

\* Based on the equivalent of 250 gm. dry soil.

available organic matter. It was thought that these factors might in part contribute to the marked differences established in regard to the behavior of the six soils. Fresh soils from the same plots were obtained 6 months after the removal of the first samples. The data included in table 7 are based on the results of study of these samples of fresh soils not submitted to the process of

air drying. These results are comparable with those of the air-dried soils and lead to the conclusion that the respiratory capacity and cellulose decomposing power of the acid soils are significantly different from those of pH 7.0 to 7.3 and therefore that soil acidity has a definite controlling influence on the biological activities of the soil as measured in terms of carbon dioxide production.

*Number of microorganisms in the six soils and the relative nitrifying capacity*

The number of microorganisms present in the limed and unlimed air-dried soils and in the fresh screened soil was determined in accordance with the procedure proposed by Fred and Waksman (1). These data, included in table 8, are based on the count at the end of 7 days of plate incubation. There is a fairly close correlation between the numbers of microorganisms, the respiratory capacity, and the organic matter decomposing power of the different soils. The effect of air drying the soils upon the stimulation of the number of micro-

TABLE 8  
*Number of microorganisms and relative nitrifying capacity of the six soils\**

SOIL NUMBER	RELATIVE† NITRIFYING CAPACITY (AIR-DRIED SOIL)	FRESH SCREENED SOIL NOT AIR-DRIED		AIR-DRIED SOIL			
		Bacteria	Fungi	Unlimed		Limed	
				Bacteria	Fungi	Bacteria	Fungi
		<i>thousands</i>	<i>thousands</i>	<i>thousands</i>	<i>thousands</i>	<i>thousands</i>	<i>thousands</i>
1	100	21,850	75	85,300	71	81,600	74.5
2	81	14,900	45	72,050	98	73,650	94
3	72	12,300	80	66,350	100	68,000	24.5
4	31	7,800	355	38,100	137	68,100	48
5	7	1,500	405	15,000	35	63,600	131
6	10	850	190	2,650	74.5	116,000	103.6

\* Per gram of soil—7-day incubation.

† Based on soil 1-100.

organisms is parallel to the influence of this factor upon the rate of decay of organic matter. The effect of lime in bringing about a change in both the total number of microorganisms of the four acid soils and the proportions of bacteria and fungi are in accordance with results of various microbiologists. These data together with those shown in the preceding tables substantiate the observations of Stoklasa (2) to the effect that the accumulation of organic matter in acid soils is due to the controlling influence of acidity upon the activity of soil microorganisms. The restoration of the acid soils to normal respiratory capacity and cellulose decomposing power through the use of lime has been shown to have been made possible by a pronounced stimulation of the number of microorganisms.

The effect of acidity upon the relative nitrifying capacity of the six soils is parallel to the rate of carbon dioxide production. These data show that available nitrogen is an important factor in determining the rate of decomposition of organic materials in acid soils. It has been shown, however, that the

applications of available nitrogen, phosphorus, and potassium failed to restore the acid soils to a normal activity in relation to the two alkaline soils.

*Cellulose decomposing power of limed and unlimed plot soils of the old fertilizer series*

Further confirmation of the data, which show that the cellulose decomposing power of soils is influenced by soil reaction and the use of lime, is given in table 9. These data show that there is a marked difference between the cellulose decomposing power of the plot soils of tier 1 and those of tier 4, treated the

TABLE 9  
*Relative cellulose decomposing power of certain field plot soils of the old fertilizer series as influenced by lime*

PLOT NUMBER	FIELD TREATMENT	REACTION OF SOILS		BASED ON THE MGM. CO <sub>2</sub> PRODUCED BY SOIL + CELLULOSE IN EXCESS OF THE SOIL ALONE	
				Soils of Tier 1 taken as 100	
		Tier 1	Tier 4	Tier 1 unlimed	Tier 4 limed
		pH	pH		
1	No treatment	5.8	7.3	100	159
9	PK + 24N (DB)	5.2	6.4	100	155
10	PK + 48N (DB)	5.1	6.1	100	139
11	PK + 72N (DB)	5.2	6.7	100	148
17	PK + 24N (DB)	5.0	6.7	100	132
19	PK + 48N (DB)	5.0	6.9	100	155
21	PK + 72N (DB)	5.1	6.6	100	127
26	PK + 24N (NaNO <sub>3</sub> )	5.3	6.5	100	145
27	PK + 48N (NaNO <sub>3</sub> )	5.4	6.4	100	125
28	PK + 72N (NaNO <sub>3</sub> )	5.5	6.4	100	140
29	PK	5.2	6.3	100	154
30	PK + 24N (NH <sub>4</sub> SO <sub>4</sub> )	4.8	6.3	100	164
31	PK + 48N (NH <sub>4</sub> SO <sub>4</sub> )	4.4	5.8	100	121

same for half a century except that the plots of tier 4 received a dressing of limestone in 1923 while the plots of tier 1 have never been limed. Limestone was applied to the plot soils of tier 4 in amounts sufficient to correct the acidity in terms of the lime requirement method of the senior author (5). The data of table 9 are expressed for convenience of study in terms of the cellulose decomposing power of each plot of tier 1 taken as 100 and are based on the total carbon dioxide produced in 14 days from 250 gm. of soil. The cellulose decomposing power is measured in terms of the carbon dioxide produced by the soil + cellulose in excess of that derived from the soil alone. A study of these data shows that the limed soils in the case of each treatment have a cellulose decomposing power greatly in excess of the unlimed soils of tier 1. A

summary of the data of table 9 based on the actual milligrams of carbon dioxide produced is as follows:

	<i>Tier 1</i>	<i>Tier 4</i>
Average mgm. CO <sub>2</sub> .....	798	1,130
Difference.....		332
Per cent increase.....		41.6

#### SUMMARY

A stock soil was prepared consisting of five parts of Hagerstown silt loam and one part each of sewage sludge and find sand. The stock soil thus prepared was divided into 10 equal portions of 260 pounds. Nine portions were treated with different amounts of sulfur, and one portion received no sulfur, and all portions were incubated in small field plots for a period of 2 years. At the end of the 2-year period, six of these soils showing the following range in pH—7.4, 7.1, 6.0, 4.5, 3.8 and 3.2 were selected for a study in which soil acidity was the one limiting factor.

The respiratory capacity and organic matter decomposing power of the six soils in relation to acidity were measured in terms of carbon dioxide produced at definite intervals in relation to various organic treatments including cellulose, manure, cotton seed meal, and starch. The influence of the addition of soluble nitrogen dipotassium phosphate, and hydrated lime was also studied. The data include results obtained from both the air-dried soils and those taken later from the small plots and incubated without air drying. The respiration studies were carried out in liter side-neck filter flasks, and the carbon dioxide was measured at 2-day intervals by absorption in a train of soda lime tubes.

The data derived from a study of the soils made acid by the oxidation of applied sulfur, were supplemented with additional studies of limed and unlimed soils which had received similar fertilizer treatment for a period of half a century.

Statistical studies of the method used for the measurement of carbon dioxide showed a probable error of  $\pm 1.95$  per cent based on 54 duplicate determinations of the total carbon dioxide produced in 14 days compared to a probable error of  $\pm 5.2$  per cent based on 378 duplicate determinations of the carbon dioxide recovered at 2-day intervals. These statistical studies formed a basis for determining the significance of the differences in behavior of the soils of different pH values with respect to the production of carbon dioxide derived from the treated and untreated soils. These studies lead to the following conclusions:

A close correlation was found to exist between the reaction of the six soils and their respiratory capacity and organic matter decomposing power.

The addition of soluble nitrogen to the soils treated with organic materials and to the soil alone caused an increase of carbon dioxide production of all six soils but failed to restore the activity of the acid soils to a degree comparable with that of the soils of pH 7.4 and 7.1.



An application of dipotassium phosphate to the soil + cellulose + nitrogen treatment had little effect either in stimulating carbon dioxide production or in changing the established ratio of the relative cellulose decomposing power of the six soils.

Lime applied to the four acid soils 30 days prior to the respiration studies followed by the cellulose + nitrogen treatment, restored the acid soil to a carbon dioxide production capacity comparable with that of the two alkaline soils.

The process of air drying the six soils greatly stimulated the production of carbon dioxide compared with the fresh screened soil. The relative respiratory capacity and cellulose decomposing power of the two sets of soils, however, were quite similar.

The use of corn starch as a source of organic matter gave results contrary to the data obtained with cellulose, manure, and cotton seed meal. It is concluded that cellulose (ground filter paper) is best adapted for such studies.

The respiratory capacity and organic matter decomposing power of the six soils run parallel with the number of soil microorganisms present, in the case both of the air-dried soils and of those incubated without air drying.

Lime stimulated both the production of carbon dioxide of the acid soils and the number of soil microorganisms.

The limed soils of the old fertilizer plots show a cellulose decomposing power 41.6 per cent in excess of that of the unlimed soils.

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## THE SETTLING VOLUME OF SOILS

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The soils of the Erosion Experiment Stations present a number of distinct soil types representative of the Great Soil Groups [Marbut's Classification (5)]. The lateritic soils are represented by the Kirvin fine sandy loam and the Nacogdoches fine sandy loam at Tyler, Texas, and the Cecil sandy clay loam at Statesville, N. C.; the prairie soils are represented by the Shelby silt loam at Bethany, Mo., the Marshall silt loam at Clarinda, Iowa, and the Palouse silt loam at Pullman, Wash.; the chernozem group is represented by the Colby silty clay loam at Hays, Kans.; the Houston black clay is a rendzina soil at Temple, Texas. The gray-brown podzolic soils are represented by the Vernon fine sandy loam at Guthrie, Oklahoma, the Knox<sup>2</sup> silt loam at La Crosse, Wisconsin, and the Muskingum silt loam at Zanesville, Ohio. Descriptions and analytical data on the Knox, Nacogdoches, and Muskingum soils have not been published. Rather full data on all the others have been published (7).

In a study of the erosional characteristics of these soils, Middleton (6) measured what he calls the "dispersion ratio," the relation between the amount of material which, under specific conditions, remains suspended in water and the total silt and clay of the soil. In the seven erosion station soils (7) this value varied for the different horizons between the limits of 5.4 and 41.4 per cent.

While these measurements were being made it was observed that when the suspended soils were allowed to stand in the presence of excess water, very different minimum volumes were reached. These volumes obviously represent a soil water equilibrium system at the maximum water content. It seemed of interest to determine this relation as exactly as possible, in view of the fact that it may be related to the erosional behavior of the soils.

The following procedure was adopted. A quantity of air-dry soil equivalent to 50 gm. of oven-dry soil is put into a 250-cc. beaker, and 35-40 cc. of water is added. The soil and water are vigorously stirred and the beaker is placed in a vacuum desiccator and evacuated until the mixture boils vigorously. The mixture is poured into a 100-cc. graduate with the minimum quantity of water

<sup>1</sup> Associate physicist and principal chemist, respectively, Division of Soil Chemistry and Physics, Soil Investigations.

<sup>2</sup> In a forthcoming technical bulletin on the erosion station soils this soil type is termed "Clinton silt loam" in order to conform to the present usage of the U. S. Department of Agriculture Soil Survey.

required for rinsing the beaker. The resulting volume is usually about 90 cc. The graduate is covered by the palm of one hand and shaken vigorously. It is then set on the table, the inside washed down with a fine water jet, and the volume made up to 100 cc. The suspension is allowed to stand for 24 hours and

TABLE 1  
*Settling volume of soils from the erosion experiment stations*

SAMPLE NO	SOIL TYPE AND LOCATION	HORIZON	SETTLING VOLUME	COLLOID BY WATER VAPOR ABSORPTION	MOISTURE EQUIVALENT	WATER SATURATION CAPACITY	APPARENT SPECIFIC GRAVITY OF SOIL IN SUSPENSION ON DRY BASIS	SPECIFIC GRAVITY OF SUSPENSION
			cc.	per cent	per cent	per cent		
6678	Kirvin fine sandy loam,	A	41	5.9	7.9	45.0	1.22	1.77
9	Tyler, Tex.	B	67½	56.6	30.5	98.8	0.74	1.47
6718	Vernon fine sandy loam,	A	44	7.4	9.6	50.2	1.14	1.71
20	Guthrie, Okla.	B	54	21.5	17.7	70.8	0.93	1.58
10362	Knox silt loam, La Crosse,	A	53	13.7	23.9	68.0	0.94	1.58
3	Wis.	B	54	20.3	23.5	71.0	0.93	1.58
9475	Nacogdoches fine sandy	A	48	18.6	14.3	59.6	1.04	1.66
6	loam, Tyler, Tex.	B	59	50.5	25.2	83.0	0.85	1.55
6977	Cecil sandy clay loam, States-	A	53	20.0	20.0	68.0	0.94	1.59
8	ville, N. C.	B	58½	45.7	26.6	80.4	0.86	1.54
6797	Shelby silt loam, Bethany,	A	55	23.1	24.5	72.0	0.91	1.56
8	Mo.	B	67	49.6	34.9	86.8	0.75	1.47
8069	Palouse silt loam, Pullman,	A	56	26.4	25.1	74.6	0.89	1.56
70	Wash.	B	61	35.6	27.8	85.4	0.82	1.52
6842	Colby silty clay loam, Hays,	A	59	32.1	27.3	80.0	0.85	1.53
	Kans.	B	60	30.7	25.2	82.6	0.83	1.52
8736	Marshall silt loam, Clarinda,	A	62	33.9	30.1	86.0	0.81	1.50
7	Iowa	B	68	39.4	31.8	98.4	0.74	1.46
6096	Houston black clay, Temple,	A	63	41.1	30.5	88.4	0.79	1.50
7	Tex.	B	62½	40.2	27.6	87.8	0.80	1.50

the soil volume noted. The graduate is again shaken and allowed to stand 24 hours. The process is repeated until a constant volume is reached; this obtains usually after three or four shakings. The volume is noted to the nearest 0.5 cc. Duplicates agree within 1 cc., and the difference is seldom so great. The line between soil suspension and water is very sharp. Occasionally there

is a suspension of colloid of small magnitude in the supernatant liquid, but, when it finally clears, the flocculated material seldom changes the volume by more than 1 cc. On long standing, indeed for several weeks, the volume of the suspension does not change materially. In some instances anaerobic fermentation produces a gas evolution which causes a slight increase in volume. Occasionally, also, a slight decrease occurs. The appearance of the settled soils from the surface horizon of eight of the erosion station soils is shown in plate 1.

The volume of soil water suspension so obtained evidently represents the maximum volume that soil can maintain "permanently" in the presence of water. It has been carefully measured for the A and B horizons of 10 soils from nine erosion stations. The results are given in column 4, table 1.

In table 1 the soils are listed in the order of the colloid content of the surface horizon, and the colloid content for both horizons, as determined by the water

TABLE 2

*Erosion ratios of the surface soils of the Erosion Experiment Stations and the apparent specific gravities of the soils in suspension*

SOIL TYPE	APPARENT SPECIFIC GRAVITY OF SOIL IN SUSPENSION ON DRY BASIS	EROSION RATIO
Kirvin fine sandy loam . . . . .	1.22	50.2
Vernon fine sandy loam . . . . .	1.14	30.3
Nacogdoches fine sandy loam . . . . .	1.04	21.7
Knox silt loam . . . . .	0.94	57.4
Cecil sandy clay loam . . . . .	0.94	22.9
Shelby silt loam . . . . .	0.91	28.8
Palouse silt loam . . . . .	0.89	19.4
Colby silty clay loam . . . . .	0.85	13.0
Marshall silt loam . . . . .	0.84	14.2
Houston black clay . . . . .	0.79	8.1

vapor absorption method, is given in column 5. The moisture equivalent is given in column 6. In column 7 is given the "water saturation capacity,"<sup>\*</sup> which is the weight of enclosed water in the settling volume of the soil expressed as the percentage of the weight of the dry soil. This is calculated by subtracting the absolute volume of the oven-dry soil (calculated from specific weight data not given in the table) from the settling volume of the soil and dividing this difference, which numerically corresponds to the weight of the water in the suspension, by the weight of the soil. The quotient  $\times 100$  gives the water saturation capacity expressed in percentage. From the data and the specific gravity may also be calculated the apparent specific gravity of the soil as suspended and the specific gravity of the suspension (columns 8 and 9).

\* Possibly this term is not well chosen because of possible confusion with "maximum water holding capacity," as used by Hilgard, and "saturated" soils, as used in base exchange studies. No better term, however, has occurred to the writers.

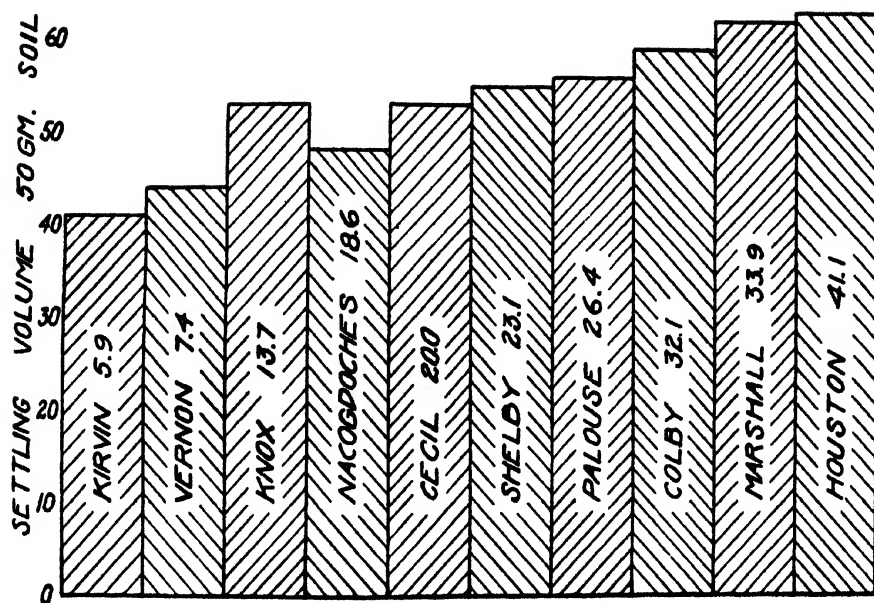


FIG. 1. SETTLING VOLUMES OF A HORIZONS

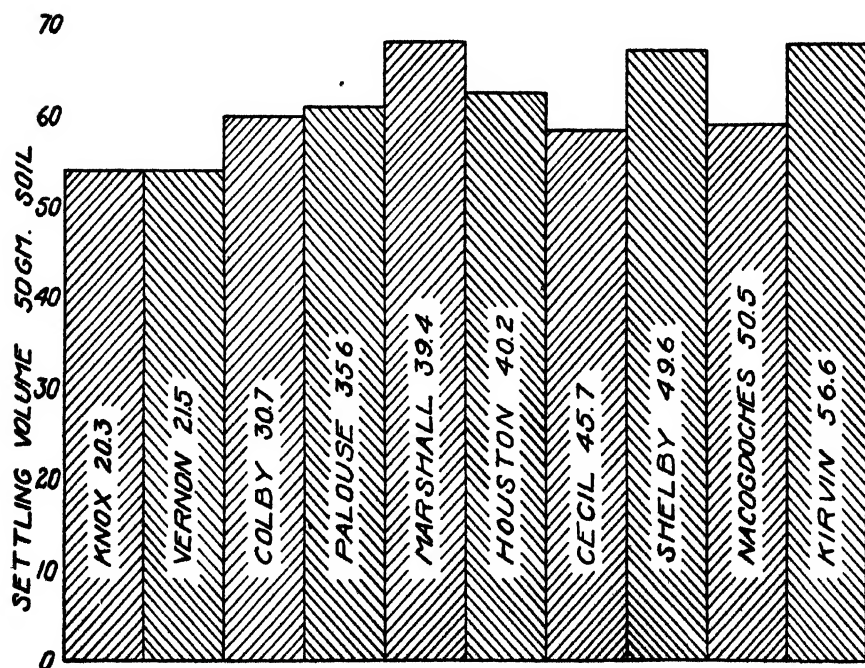


FIG. 2. SETTLING VOLUMES OF B HORIZONS

It might be expected that a certain relation might exist between the erosion ratio (3) and the apparent specific gravity of the soil in suspension; assuming that runoff liquid consists of soil at its water saturation capacity, it would follow that the greater the apparent specific gravity of the soil suspension, the greater the loss of soil per unit of runoff. That there is a qualitative relation may be seen by comparing the erosion ratios of these soils (7) with the quantities given in column 7 as shown in table 2. This relation in itself leaves much to be desired, though in general the high erosion ratios are found for soils with apparent high specific gravity. If we make the same comparison for the B horizon the same trend appears, but with even more erratic variations.

A more satisfactory relation appears when we compare the settling volume (table 1) with the colloid percentage, as is done graphically in figure 1.

TABLE 3  
*Mechanical composition and settling volumes of A horizons of the erosion station soils\**

SOIL TYPE	MECHANICAL ANALYSIS†			SETTLING VOLUME		DIFFERENCE
	Sand	Silt	Clay	Observed	Calculated	
	per cent	per cent	per cent	cc.	cc.	cc.
Kirvin fine sandy loam.....	75.8	15.7	8.6	41	43.8	+2.8
Vernon fine sandy loam.....	73.3	18.5	8.1	44	44.0	0
Nacogdoches fine sandy loam.....	69.7	9.8	18.0	48	45.5	-2.5
Cecil sandy clay loam.....	53.1	20.7	26.2	53	51.4	-1.6
Shelby silt loam.....	26.0	46.0	27.9	55	55.9	+0.9
Palouse silt loam.....	6.4	64.0	27.5	56	57.7	+1.7
Colby silty clay loam.....	18.5	46.5	35.0	59	58.7	-0.3
Marshall silt loam.....	2.1	59.0	35.3	62	59.4	-2.6
Houston black clay.....	10.9	26.0	60.4	63	64.4	+1.4

\* Knox silt loam had not been received when this portion of the work was completed.

† Determinations by H. W. Lakin and T. M. Shaw.

In this figure the soils are arranged in the order of their colloid content, and the settling volume is charted to scale. With the single exception of the Knox silt loam, the colloid content corresponds qualitatively with the settling volume. This relation is to be expected, since the volume of water absorbed is, as in general moisture relations, greatly affected by colloid content. However, if we make a corresponding chart of the B horizon relations, this harmony of relationship quite disappears (fig. 2). The Shelby soil, with 49.6 per cent colloid, has a slightly smaller settling volume than the Marshall, with 39.4 per cent colloid, whereas the Nacogdoches soil, with essentially equal settling volume with the Cecil and 5 per cent more colloid, has a slightly smaller settling volume than the Colby, with 20 per cent less colloid. Clearly, therefore, the settling volume is not wholly a colloidal effect.

The moisture equivalent is a soil relation which is primarily dependent upon

colloidal content but which, as was shown by Briggs and McLane (3), is partly dependent upon the mechanical components, as expressed by the following equation:

$$0.13 C + 0.62 (D + E) = H \pm 3,$$

where  $C$  is the silt content and  $D$  and  $E$  the clay and organic contents, respectively.

In a similar manner the method of least squares applied to the data given in table 3 gives the equation for the surface soils:

$$0.38 \times \text{sand} + 0.53 \times \text{silt} + 0.77 \times \text{clay} = \text{settling volume} \pm 1.29$$

TABLE 4  
*Mechanical composition and settling volume of B horizons of the erosion station soils*

SOIL TYPE	MECHANICAL ANALYSIS*			SETTLING VOLUME		DIFFERENCE
	Sand	Silt	Clay	Observed	Calculated†	
	per cent	per cent	per cent	cc.	cc.	cc.
Kirvin fine sandy loam.....	31.1	7.4	61.5	67½	63.0	-4.5
Vernon fine sandy loam.....	60.3	12.4	27.2	54	50.4	-3.6
Nacogdoches fine sandy loam.....	45.7	4.9	48.4	59	57.2	-1.8
Cecil sandy clay loam.....	30.8	12.1	57.1	58½	62.0	+3.5
Shelby silt loam.....	19.4	27.7	52.9	67	62.8	-4.2
Palouse silt loam.....	5.9	55.6	37.5	61	60.7	-0.3
Colby silty clay loam.....	18.8	42.8	38.5	60	59.6	-0.4
Marshall silt loam.....	1.5	56.5	39.4	69	61.0	-7.0
Houston black clay.....	9.6	25.1	64.1	62½	66.3	+3.8

\* Determinations by H. W. Lakin and T. M. Shaw.

† Calculated by formula for A horizons.

If these quantities are used to calculate the settling volume of the soils the values given in column 6 are obtained and the differences from the values found are given in column 7. This relation is quite satisfactory if taken alone, but becomes much less satisfactory when applied to the corresponding data for the B horizons, as shown in table 4.

If, on the other hand, the distribution is calculated from the data in table 4, the formula becomes.

$$0.45 \times \text{sand} + 0.61 \text{ silt} + 0.72 \times \text{clay} = \text{settling volume} \pm 2.23$$

This result, even for the data in table 4, is not very satisfactory and becomes even less coherent when applied to the surface soils.

It would seem, however, that the soil characteristics which are responsible for the moisture equivalent should be those which produce the settling volume differences. Figure 3 represents an attempt to correlate the moisture equivalent and settling volume relations.

It will be seen that a reasonably close relation obtains for the surface horizons. The relation for the B horizons is less satisfactory. It is clear, then, that other factors than texture enter into the determination of the settling volume, though texture would seem most important.

What part is played by organic matter is difficult to determine, though the soils examined vary widely in organic content. The organic content of the A and B horizons for the 10 soils is given in table 5.

Those soils high in organic matter are usually high in clay content and in colloid content. They are also high in settling volume, but it seems that the effect of organic matter, if any, is masked by the variations in clay or colloid content.

It seems probable that the settling volume should also be influenced by the character as well as by the content of colloid. To determine whether this is the case, the settling volume of the colloids themselves was determined in

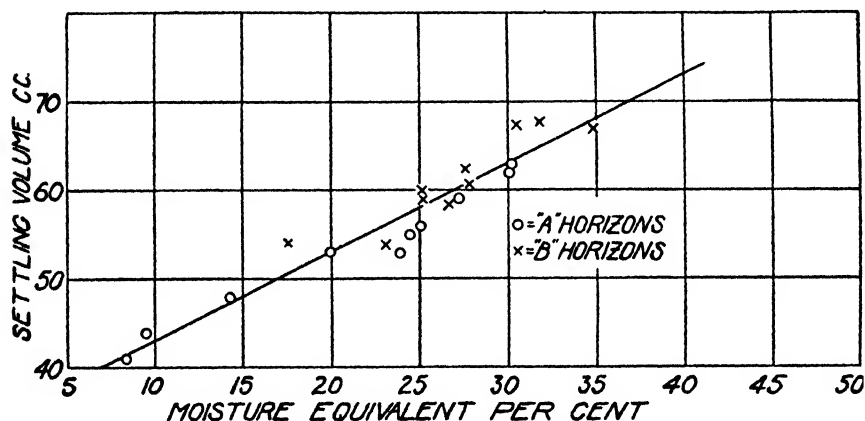


FIG. 3. RELATION OF SETTLING VOLUME TO MOISTURE EQUIVALENT

exactly the same manner as with the soils, except that 10 gm. of colloid were used. The quantity of colloid used is not an important factor, as was shown by using 5, 10, and 25 gm. of the Shelby silt loam colloid with the same volume of water. The settling volumes obtained were 11, 21½, and 53 cc., respectively. It will be seen from these figures that larger quantities of colloid give slightly lower settling volumes per unit weight in this case. Sufficient quantities of colloid were not readily available in the case of the other soil types. The variation, however, is not great, and the comparisons based upon the use of 10 gm. of colloid were considered adequate for the purpose in hand.

The colloids were extracted from the soils, without any dispersing agent, were air dried and rolled to pass a 2-mm. sieve. The determinations were more difficult and less satisfactory than in the case of the soils, since there is a tendency for more of the material to remain in colloidal suspension in all but the carbonate containing colloids. The settling volumes were therefore more



affected by the "volume of floc" as a result of slow flocculation. There was in all cases considerable flocculation. The results obtained are given in table 6.

In this table the colloids are listed in the order of their silica-sesquioxide ratios, which are also given in column 4. In general, it will be observed that the settling volume increases as the silica-sesquioxide ratio becomes greater.

TABLE 5  
*Organic content of erosion station soils\**

SOIL SERIES	A HORIZON	B HORIZON
	<i>per cent</i>	<i>per cent</i>
Shelby.....	3.23	2.17
Marshall.....	3.22	2.29
Cecil.....	3.21	0.45
Houston.....	2.94	1.88
Colby.....	2.82	1.11
Palouse.....	2.18	0.96
Nacogdoches.....	1.89	1.13
Vernon.....	1.81	0.62
Knox.....	1.55	0.59
Kirvin.....	0.54	0.81

\* Determinations by C. S. Slater.

TABLE 6  
*Settling volumes of colloids extracted from the A horizons of the erosion station soils\**

SOIL FROM WHICH COLLOID WAS EXTRACTED	SETTLING VOLUME	SPECIFIC GRAVITY	SILICA-SES- QUOXIDE RATIO†	WATER SATURA- TION CAPACITY	APPARENT SPECIFIC GRAVITY OF COLLOID IN SUSPENSION ON DRY BASIS
	<i>cc.</i>			<i>per cent</i>	
Nacogdoches fine sandy loam.....	16	2.92	1.07	126	0.63
Cecil sandy clay loam.....	19	2.69	1.34	153	0.52
Kirvin fine sandy loam.....	18½	2.75	2.02	147	0.54
Palouse silt loam.....	22	2.69	2.48	183	0.45
Shelby silt loam.....	21½	2.64	2.65	177	0.47
Knox silt loam.....	22½	2.60	2.69	187	0.44
Marshall silt loam.....	22	2.62	2.91	182	0.45
Houston black clay.....	26	2.67	3.26	223	0.38
Colby silty clay loam.....	24½	2.67	3.45	208	0.41

\* Vernon fine sandy loam was omitted because of lack of sufficient sample.  
Determinations by C. S. Slater.

The relation is not quantitative. The water saturation capacity and the apparent specific gravity, which are quantities derived from the settling volume, follow the same general relation—the one directly and the other inversely.

It is evident that these colloid-water relations are influenced by the compo-

sition of the colloid. That this influence is marked is shown graphically in figure 4.

The three lateritic colloids, the Nacogdoches, Cecil, and Kirvin, have low settling volumes; the prairie colloids group themselves close together; the Knox colloid is from a soil developed in a prairie region, though apparently under forest cover. The two colloids of greatest settling volume both contain calcium carbonate, but also have the highest silica-sesquioxide ratios of the group of soils examined.

That a relation between the composition and the water relationships of soil colloids exists has been pointed out by Anderson and Mattson (2) and the nature of the differences in composition giving rise to these differences in behavior has been discussed by Byers (4). That the relation is not quantitative is to be expected in the light of the data given by Anderson (1) for a series of

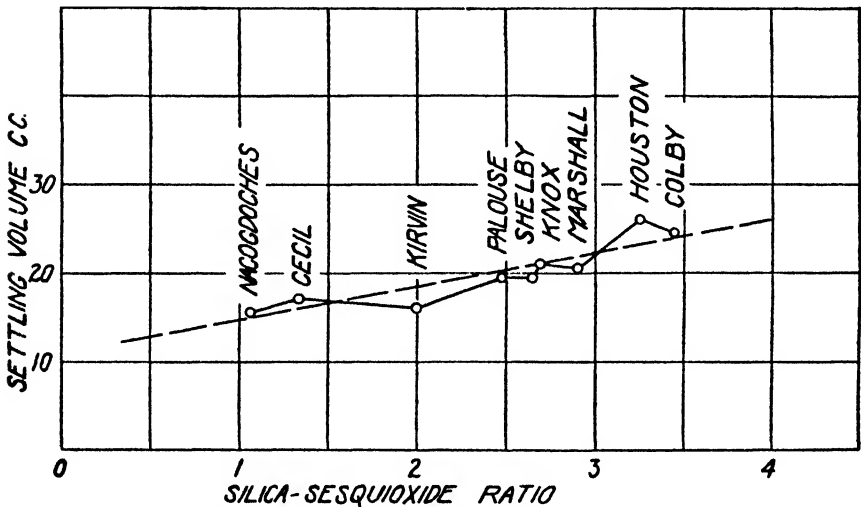


FIG. 4. RELATION OF SETTLING VOLUME OF COLLOIDS TO SILICA-SESQUIOXIDE RATIO

soil colloids ranging in silica-sesquioxide ratios from 0.31 to 3.62. In these colloids marked influence upon the moisture equivalent, swelling, water vapor absorption, and heat of wetting was shown by the bases associated with the colloid.

In the case of the colloids from the erosion experiment stations, the exchangeable bases and the base exchange capacities have not been determined. It is known, however, that these colloids range from the high calcium, high pH, and saturated chernozems to the highly leached lateritic soils. Whether it is possible eventually to trace the extent of the influence of these various factors upon the water relationships of the soils is not yet known.

The investigation of these related properties is, of course, being continued. In the meantime, the relationships shown by the graphs in figure 5 are of considerable interest.

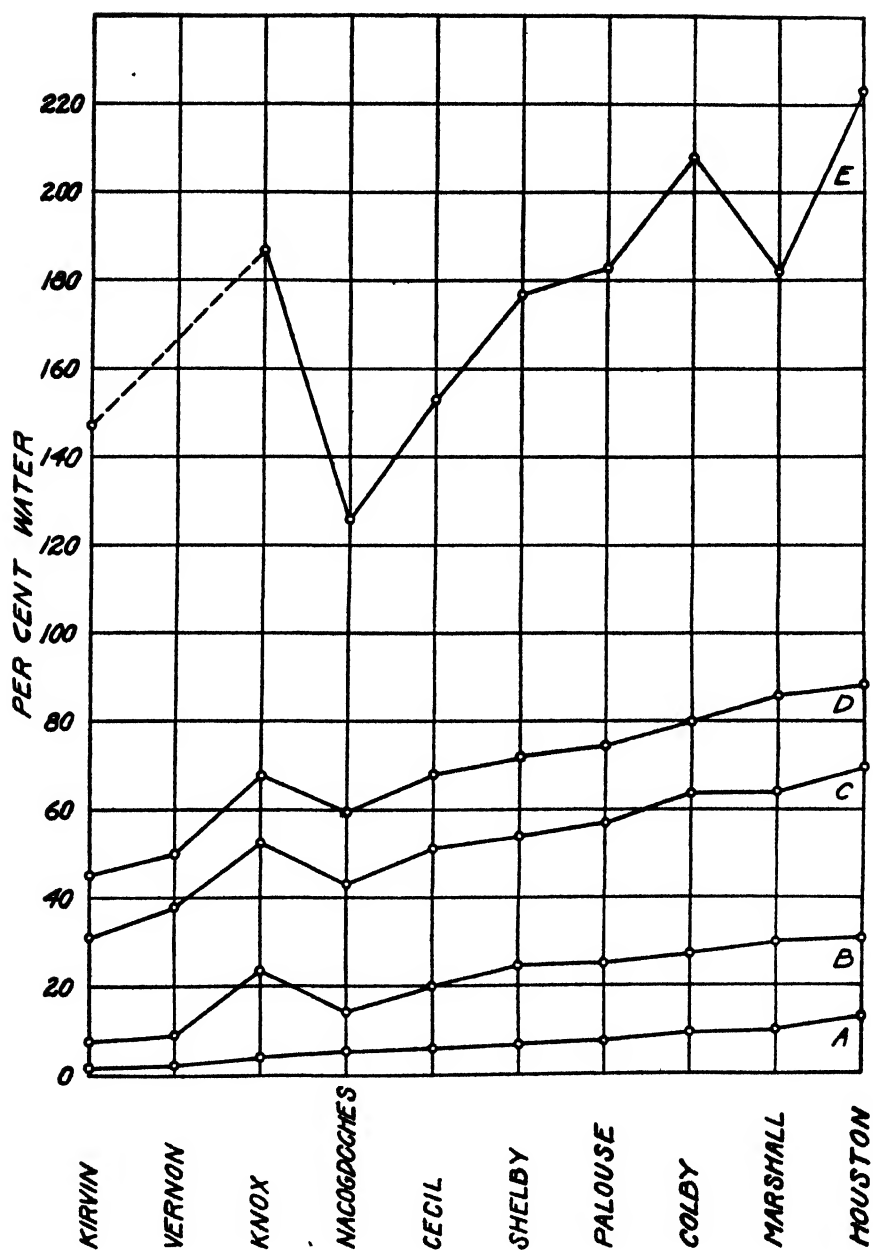


FIG. 5. WATER RELATIONSHIPS OF EROSION STATION SURFACE SOILS

- A. Water vapor absorption.
- B. Moisture equivalent.
- C. Maximum water holding capacity.
- D. Water saturation capacity.
- E. Water saturation capacity of colloid fraction.

In these graphs the soils are placed, at regular intervals, in the order of their colloid content. The relationship is not quantitative. That the composition of the colloid and the texture of the soil influence all the soil moisture relationships is clear. Perhaps the most interesting thing about the graphs is the abnormal values shown by the Knox silt loam soil with respect to all the properties shown, except the water vapor absorption. That this abnormality is due to the colloid, the water saturation capacity of the colloid clearly shows. Why this colloid, present in relatively small amounts, should be of such marked influence upon the soil behavior is also clearly owing to its silica-sesquioxide ratio, as indicated by the graph shown in figure 4.

We have no clue at present to the inverted relationship of the Colby and Houston colloids and soils. That the soil-water relationship which we have called the "settling volume of soils" is a real and reproducible soil property is made abundantly clear by this figure and the data from which it is developed.

#### SUMMARY AND CONCLUSIONS

A new soil-water and a new soil colloid-water relation is defined and a method for its determination is described. The relation of this soil property to the colloid, silt, and clay content is discussed, as well as its relation to colloid, composition, organic matter content, and the erosion ratio.

This soil-water relation, the settling volume of soils, is measured for 10 soils of widely different character. The soils are from the Erosion Experiment Stations and their general chemical and physical properties are known.

The term "water saturation capacity" is used to define the percentage water content of the soils at their settling volume, and a comparison is made between this value and the water vapor absorption, moisture equivalent, and total maximum water holding capacity of the soils. It is pointed out that this relation, as far as it is dependent upon colloid composition, is affected not only by the character of the acids and oxides present, and by the organic matter, but also by the kind and quantity of bases present.

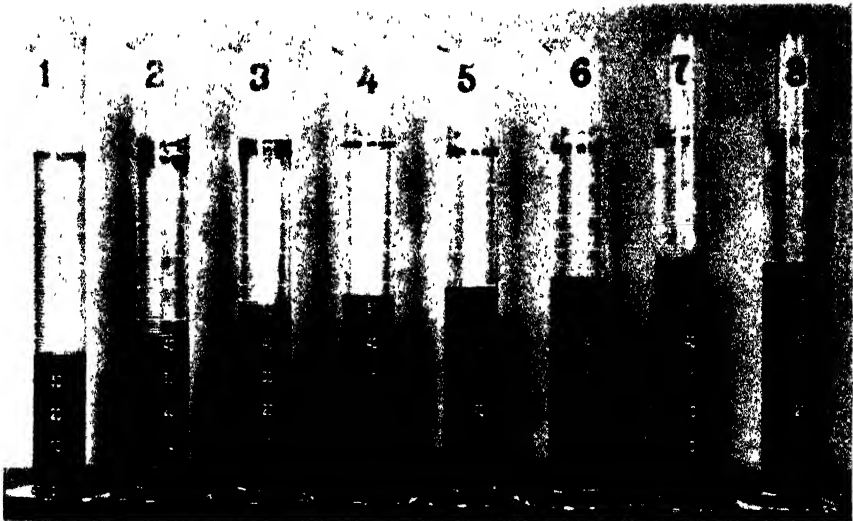
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## PLATE 1

## SETTLING VOLUMES OF A HORIZONS

1. Kirvin fine sandy loam
2. Vernon fine sandy loam
3. Cecil sandy clay loam
4. Shelby silt loam
5. Palouse silt loam
6. Colby silty clay loam
7. Marshall silt loam
8. Houston black clay





# AN EXAMINATION OF THE DEGTJAREFF METHOD FOR DETERMINING SOIL ORGANIC MATTER, AND A PROPOSED MODIFICATION OF THE CHROMIC ACID TITRATION METHOD

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Among the many methods of measuring the carbon content of soils two main groups may be recognized: those methods which determine the carbon dioxide produced by oxidation either in the wet or in the dry way, and those which in effect measure the reducing power of the soil by some oxidizing agent. Of the first type of method probably the only one which can be relied upon to give accurate results is the dry combustion method of Liebig or its modification by Dennstedt (3, 4, 5). The various types of wet combustion using chromic acid, though superior in accuracy to most of the methods based on reduction, frequently fail to give the complete oxidation usually claimed for them. The second or indirect type of method is based upon the assumption that carbon is the chief reducing agent present in soils and that any other substance exerting a minor effect will bear a constant proportion to the carbon. Any hydrogen atoms in the organic complex not fully oxidized already are likely to undergo oxidation along with the carbon, whereas nitrogen atoms, in taking up hydrogen to form ammonia, will act in the opposite way, so that one must assume that the proportions of carbon, oxidizable hydrogen, and reducible nitrogen are present in the organic matter of soils in about the same proportions. There is also the possibility of the presence of inorganic substances of an oxidizing or reducing nature, though probably these do not commonly occur.

Since indirect methods rarely give complete oxidation, we must make the further assumption that there is a definite fraction of organic matter common to many different kinds of soil which is readily attacked by oxidizing agents. In support of this there are the results of W. McLean (6), who has shown that 3 per cent hydrogen peroxide removes 85 per cent of the organic matter from a number of soils. Oxidations with sodium hypobromite and with chromic acid behave similarly.

Indirect methods may thus prove useful in the rapid examination of large numbers of soil samples for survey or advisory work when the necessary factor has been established by more exact determinations on typical ones. In the course of a comparative study by one of us on a number of such rapid methods,

<sup>1</sup> The authors wish to express their thanks to Dr. E. M. Crowther for a number of valuable suggestions and for his helpful criticism throughout.



the one proposed by Degtjareff (2) was carefully examined, for we could find no theoretical basis for its action.

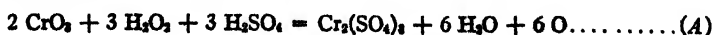
In the Degtjareff method 0.15 to 0.2 gm. finely ground soil is treated first with 10 or 15 cc. of 0.3 per cent hydrogen peroxide and then with an equal volume of a solution of 1.6 per cent chromic acid in concentrated sulfuric acid. Considerable heat is evolved. The mixture is shaken for a minute, washed into a beaker, and diluted to about 200 cc. Excess chromic acid is then titrated with ferrous ammonium sulfate in the presence of diphenylamine and from the amount of chromic acid reduced the carbon content of the soil is calculated.

The form of the titration, originally suggested by Schollenberger (8) was subsequently improved by him (9) by the addition of sodium fluoride, which makes the end point much sharper by preventing the buffering action of iron salts. We have used throughout Schollenberger's improved method of titration.

Since chromic acid and hydrogen peroxide react with mutual decomposition it is difficult to see what useful function the peroxide can perform other than that of providing water with which to generate heat when mixed with the strong sulfuric acid solution. It necessarily reduces the strength of the chromic acid, which is in excess, and if any organic matter is oxidized by the hydrogen peroxide or by nascent oxygen from its decomposition, the net result must be to reduce the apparent carbon content, since less chromic acid will then be used up.

With the short interval of contact between soil and the oxidizing agent it seemed most unlikely that oxidation of soil carbon could be complete. It thus appeared probable that some concealed error happened to cancel out the incompleteness of oxidation under the conditions used by Degtjareff. Preliminary trials soon showed that the apparent carbon content fell off rapidly when increasing amounts of soil were used in the determination. Since the work here reported was completed we have received a paper in which Tiurin (10) condemned the Degtjareff method and suggested that the reaction between hydrogen peroxide and chromic acid was incomplete but proceeded further in the presence than in the absence of soil. He produced, however, no direct evidence that any hydrogen peroxide remained in the presence of excess chromic acid, and it can easily be demonstrated that in such solutions no hydrogen peroxide does in fact remain. Ether extracts a deep blue perchromic acid from chromic acid solutions containing hydrogen peroxide, but fails to do so from the Degtjareff solutions (whether with or without soil).

Degtjareff and Tiurin assume that the reaction between hydrogen peroxide and chromic acid in strong sulfuric acid is



The nature of the corresponding reaction in *dilute* acid solutions was the subject of active investigations and discussions about 30 years ago (1, 7). Equation A was generally accepted, but an alternative one



was also proposed. The reactions in dilute solutions are naturally no guide to those in strong sulfuric acid solutions, but the possibility of altering the reaction by slight changes in conditions required examination. Our work indicates that soil acts catalytically and leads to the unusual situation that one reaction proceeds in the presence of soil and the other in the blank. The difference between the two parallel determinations with and without soil therefore fails to measure the carbon content of the soil, and it is an odd chance that the excess apparent carbon given by this change in the nature of the reaction should balance out the incompleteness of the actual attack on soil carbon for the conditions under which Degtjareff appears to have worked.

#### EXPERIMENTAL

The data quoted in this paper refer to determinations made on a standard series of seven representative British Soils selected by the Soil Analysis Subcommittee of the Agricultural Education Association for coöperative work. All samples were ground in either a porcelain or an agate mortar to pass a sieve with 100 meshes to the linear inch. (It may be mentioned that in any measurement of soil organic matter by reduction of an oxidizing agent such as chromic acid or sulfuric acid, very serious errors may be introduced, especially in sandy soils, if at any stage in the preparation of the sample an iron or steel mortar and pestle are used. Sufficient metallic iron is introduced into the sample to cause reduction comparable with that of a considerable amount of carbon in soil organic matter.)

The Dennstedt (3, 4, 5) method of combustion was used as the standard method for determining organic carbon. The soil was heated in a stream of oxygen, the products of oxidation passing over a heated sheet of platinum to ensure complete oxidation and then over lead peroxide to remove oxides of nitrogen and of sulfur and halogens. The carbon dioxide produced was determined gravimetrically. Inorganic carbon (as calcium carbonate) was present in only one (Roihamsted) of the seven soils. It was removed before the combustion by evaporating the soil to dryness on a water bath with excess of a solution of sulfurous acid. This removed carbonates and left a certain amount of sulfite which was oxidized to sulfur trioxide and absorbed by the lead peroxide in the Dennstedt combustion tube.

No sulfurous acid treatment was needed for the Dennstedt combustions on the six carbonate-free soils or for any of the experiments with chromic acid, for these all measure the reduction of the chromic acid and not the carbon dioxide produced in the course of the reduction.

The details as to strength and preparation of solutions used in the reduction methods are as described by Degtjareff and are given in the following:

- (a) Hydrogen peroxide—0.3 per cent.
- (b) Chromic acid—16 gm.  $\text{CrO}_3$  is dissolved in a liter of conc.  $\text{H}_2\text{SO}_4$  and the mixture heated to  $165^\circ\text{C}$ . for half an hour to stabilize it.

(c) Diphenylamine—0.5 gm. dissolved in 100 cc. conc.  $\text{H}_2\text{SO}_4$  and 20 cc. water.

(d) Ferrous ammonium sulfate—0.2 *N*.

In each of the determinations by the reduction methods 0.15 gm. of 100-mesh soil was put into a 350-cc. flask into which was then pipetted 10 or 20 cc. of  $\text{H}_2\text{O}_2$ , followed immediately by the same volume of chromic acid solution. The mixture after having been shaken for a minute was cooled and diluted to 150:200 cc. Next, 5 gm. sodium fluoride was added and when dissolved titrated with ferrous ammonium sulfate in the presence of 1 cc. of diphenylamine. Blank experiments were carried out under identical conditions.

Table 1 gives the results of the determinations by the Dennstedt method, by the Degtjareff method using two different amounts of reagents, and also by chromic acid with water instead of hydrogen peroxide.

The results of table 1 are plotted in figure 1. It is clear that the Degtjareff method gives excessively high values for low amounts of carbon and progres-

TABLE 1  
*Carbon determinations by the Dennstedt and Degtjareff methods*

SOIL	WOBURN	HARPER ADAMS	ROTH- AMSTED	COCKLE PARK	BANGOR	CRAIB- STONE	INSCH
Per cent carbon by dry (Dennstedt) combustion.....	0.87	0.91	1.87	1.98	3.06	3.88	4.88
Per cent carbon by reduction method using:							
1. 20 cc. $\text{H}_2\text{O}_2$ + 20 cc. $\text{CrO}_3$ .....	1.78	1.73	2.89	2.45	3.32	3.97	4.55
2. 10 cc. $\text{H}_2\text{O}_2$ + 10 cc. $\text{CrO}_3$ .....	1.06	1.17	1.96	1.74	2.58	2.95	3.28
3. 20 cc. water + 20 cc. $\text{CrO}_3$ .....	0.60	0.73	1.47	1.41	2.24	2.62	3.50

sively decreasing recoveries with increasing amounts of carbon. The apparent recovery reaches 100 per cent for 0.15 gm. of a soil containing about 4 per cent of carbon when 40 cc. of mixed reagents are used in the determination.

If  $x$  represents the percentage of carbon found by combustion and  $a$  the apparent percentage of carbon by the Degtjareff method using 0.15 gm. of soil with 20 cc. of hydrogen peroxide and 20 cc. of chromic acid solution, then the regression of  $a$  on  $x$  is found to be

$$a = 0.70x + 1.22$$

Similarly if  $b$  is the apparent carbon content when 0.15 gm. of soil is used with 20 cc. of water and 20 cc. of chromic acid solution then

$$b = 0.69x + 0.07$$

These two lines are plotted in figure 1. They show that the reduction of chromic acid corresponds to approximately 70 per cent of the carbon present in the soil together with a very large constant for the determinations with

hydrogen peroxide and with a trivial one for those with water instead of hydrogen peroxide. With 40 cc. of mixed reagents for 0.15 gm. of soil, *the Degtjareff method would give an apparent carbon content of 1.2 per cent even if the soil were completely devoid of carbon.*

The determinations with smaller amounts of hydrogen peroxide and chromic acid give results about midway between those just considered, when the soils have little carbon. For the soils rich in carbon the recovery falls off rapidly, since only very small amounts of chromic acid remained available for oxidation

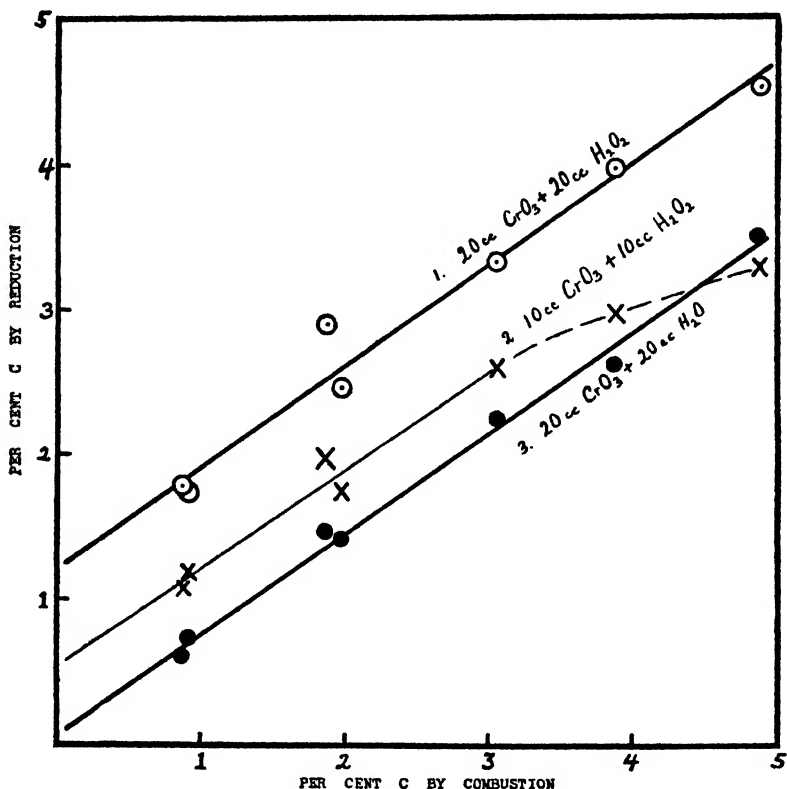


FIG. 1

after the joint reducing actions of the hydrogen peroxide and the large amount of carbon. With these smaller amounts of reagents a soil devoid of carbon would have an apparent carbon content of about 0.6 per cent.

It is clear from these results that the method as recommended by Degtjareff can have no value as an analytical method, and it becomes of interest to examine more closely the cause of its failure.

An attempt was therefore made to determine the quantitative relationships existing in the interaction of  $\text{H}_2\text{O}_2$  and  $\text{CrO}_3$  at the concentrations recommended by Degtjareff both in the presence and absence of soil.

This was done in the following manner:

- (a) 10 cc. of chromic acid solution was titrated with ferrous ammonium sulfate.
- (b) 10 cc. of chromic acid was mixed with 10 cc. of hydrogen peroxide and the excess chromic acid titrated as in (a).
- (c) The strength of the hydrogen peroxide was determined by adding to 10 cc. an excess of acidified ferrous ammonium sulfate and titrating back with potassium dichromate.

Table 2 gives the ferrous iron equivalents of the chromic acid (a), the hydrogen peroxide (c), and the excess chromic acid (b) in the mixture of chromic acid and hydrogen peroxide.

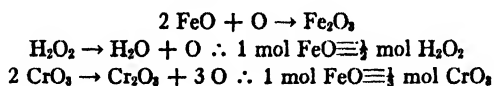
To obtain the molecular proportions reacting, these equivalents in terms of iron must be divided by 2 for the hydrogen peroxide, and by 3 for the chromic

TABLE 2

*Ferrous iron equivalents of chromic acid, hydrogen peroxide, and excess chromic acid in chromic acid and hydrogen peroxide mixture*

	TITRATION VALUE IN CC 0.2 <i>N</i> FERROUS AMMONIUM SULFATE	TITRATION VALUE EXPRESSED AS MILLIMOLS FeO	EQUIVALENT IN MILLIMOLS
(a) 10 cc. CrO <sub>3</sub> .....	18.25	3.65	1.22 CrO <sub>3</sub>
(b) 10 cc. H <sub>2</sub> O <sub>2</sub> + 10 cc. CrO <sub>3</sub> .....	11.80	2.36	0.79 CrO <sub>3</sub>
(c) 10 cc. H <sub>2</sub> O <sub>2</sub> .....	8.65	1.73	0.86 H <sub>2</sub> O <sub>2</sub>

acid, as can be seen by considering the following equations, which represent the fundamental reactions occurring:



Then 0.86 mols H<sub>2</sub>O<sub>2</sub> reacted with 1.22 - 0.79 = 0.43 mols CrO<sub>3</sub>, i.e. 2 mols CrO<sub>3</sub> reacted with 4.0 mols H<sub>2</sub>O<sub>2</sub>. A subsequent independent determination gave the ratio as 2:3.8.

In these cases the peroxide was added to the chromic acid (which was in excess). When the reagents were mixed in the reverse order (as in the Degtjareff method) there was a temporary excess of peroxide in the early part of the reaction, which might possibly be expected to result in a ratio considerably less than 2 CrO<sub>3</sub>:4H<sub>2</sub>O<sub>2</sub>. The ratio found, however, was 2:4.2.

In the absence of soil—therefore the reaction appears to be substantially that represented by the equation



When the hydrogen peroxide and the chromic acid were mixed in the presence of ignited soil it was found that the same amount of peroxide reduced more

chromic acid, as shown in table 3. Here 0.84 mols  $\text{H}_2\text{O}_2$  reacted with  $1.10 - 0.55 = 0.55$  mols  $\text{CrO}_3$ , i.e. 2 mols  $\text{CrO}_3$  reacted with 3.1 mols of  $\text{H}_2\text{O}_2$  in the presence of ignited soil as contrasted with 4.0 mols of  $\text{H}_2\text{O}_2$  in the absence of soil.

With ignited soil, then, the reaction may be represented by the equation



which was assumed by Degtjareff and Tiurin to hold both for the blank and for the actual determination on soil.

In a blank determination similar to that in table 2 and following equation A, the aforementioned amounts of reagents would react so that 1.10 millimols of  $\text{CrO}_3 + 0.84$  millimols of  $\text{H}_2\text{O}_2$  leave  $1.10 - \frac{1}{2}(0.84) = 0.68$  millimols of  $\text{CrO}_3$  instead of 0.55 millimols of  $\text{CrO}_3$ . This difference, 0.13 millimols  $\text{CrO}_3$  corresponds to an apparent carbon content of the ignited soil of 0.8 per cent and is

TABLE 3

*Ferrous iron equivalents of chromic acid, hydrogen peroxide, and chromic acid, hydrogen peroxide, and ignited soil mixtures\**

	TITRATION VALUE IN CC 0.2N FERROUS AMMONIUM SULFATE	TITRATION VALUES EXPRESSED AS MILLIMOLS FeO	EQUIVALENT IN MILLIMOLS
(a) 10 cc. $\text{CrO}_3$ .....	16.50	3.30	1.10 $\text{CrO}_3$
(b) 0.15 gm. ignited soil + 10 cc. $\text{H}_2\text{O}_2$ + 10 cc. $\text{CrO}_3$ .....	8.25	1.65	0.55 $\text{CrO}_3$
(c) 10 cc. $\text{H}_2\text{O}_2$ ...	8.45	1.69	0.84 $\text{H}_2\text{O}_2$

\* The hydrogen peroxide and the chromic acid solutions used here were not those mentioned in table 2, but differed only slightly in concentration.

thus of the same order as the figure 0.6 per cent for the apparent carbon content of a soil devoid of carbon as obtained by extrapolation from the middle curve in figure 1.

The error caused by ignited soils appeared to be almost independent of the amount of soil taken.

Thus, a series with 0.07, 0.15, and 0.30 gm. of ignited soil with 20 cc. of mixed reagents gave excess chromic acid equal to 2.0, 2.1, and 2.2 cc. respectively of 0.2 N ferrous ammonium sulfate solution over and above the true blank. A little ferric oxide gave excess chromic acid equal to 1.7 cc.

The agreement between the values calculated from soils and those found directly for ignited soils is close enough to establish the disturbance of the reaction by soil, whereas the fluctuations in the actual value of the excess are sufficiently great to invalidate the Degtjareff method even if an attempt were made to improve it by allowing, first, for the essential difference between the reactions in the presence and the absence of soil, and second, for the incompleteness of the actual oxidation.

## A PROPOSED MODIFICATION OF THE CHROMIC ACID TITRATION METHOD

From the parallelism of the lines for carbon recoveries in figure 1 it is clear that the oxidation of soil carbon proceeds just as far with chromic acid and water as with chromic acid and hydrogen peroxide and in addition that the line for the recovery with chromic acid and water passes so close to the origin that the apparent carbon contents are proportional to the true carbon contents. Although it is clearly impossible to base a rapid method on the use of chromic acid plus hydrogen peroxide, it should be possible to do so by the chromic acid plus water method provided it can be shown that the fraction of the total carbon recovered is reasonably constant for the groups of soil tested. Evidence for this possibility is afforded by Schollenberger's original proposal to determine carbon by treating a small amount of soil with 2 per cent potassium dichromate in strong sulfuric acid and heating to 175°C. in 90 seconds; by the work of Vassiliev (11), who used a slight modification of Schollenberger's method; and by Tiurin's method of treating the soil for 5 minutes with a boiling solution of chromic acid in 1:1 sulfuric acid and water. A considerable simplification in such methods may be effected by using the heat of reaction between sulfuric acid and water to avoid the necessity of external heating. This was the essential feature of Degtjareff's proposal though the principle was obscured and the method invalidated by using dilute hydrogen peroxide instead of water. There is a considerable advantage, however, in replacing the chromic acid-sulfuric acid solution, which is somewhat unstable, troublesome to make up, and difficult to measure out accurately, by aqueous potassium dichromate, which is free from these disadvantages. The necessary heating and acidity are obtained by adding sulfuric acid. Further work on the details of the method and on the significance of the readily oxidized fraction is in progress. In its present form the method is as follows. Finely divided soil, passing a 100-mesh sieve, is taken in amounts containing between 10 and 25 mgm. of carbon and placed in a 350-cc. conical flask. About 10 cc. of *N* potassium dichromate is then added from a burette, followed by 20 cc. of strong sulfuric acid, which is run in from an automatic pipette. The mixture is shaken for about 1 minute. After it has been cooled and diluted to about 150 cc., 5 gm. of sodium fluoride is added and the chromic acid remaining is titrated with 0.4 *N* ferrous ammonium sulfate, 1 cc. of  $\frac{1}{2}$  per cent diphenylamine being used as indicator. Should the end-point be overshot, the mixture can be back-titrated with the potassium dichromate solution used as oxidant.

For a series of 20 British and foreign soils examined by this procedure about 76 per cent of the carbon was accounted for by the reduction of chromic acid. The actual recoveries ranged from 60 to 86 per cent, the standard error being  $\pm 5.6$  per cent. A correction factor of  $100/76 = 1.32$  must thus be used (1 cc. *N* potassium dichromate equals  $1.32 \times 3.0 = 4.0$  mgm. carbon).

Apart from the possible practical value of such an extremely rapid and simple titration method for approximate determinations of soil carbon, it is of

interest to have additional evidence that in soils of similar types the readily oxidizable organic matter forms an approximately constant fraction of the total organic carbon. In addition, the course of the oxidation of organic matter seems to be substantially the same for all of the British soils already discussed, since direct determinations of the products of oxidation showed that about 60 per cent of the total carbon went to carbon dioxide and about 3 per cent to carbon monoxide. This would suggest the almost complete oxidation of a group of compounds common to all of these soils.

#### SUMMARY

The chromic acid-hydrogen peroxide method of Degtjareff for the rapid determination of soil carbon is shown to give entirely fictitious results.

The hydrogen peroxide not only serves no useful purpose but introduces a fundamental error, since its reaction with chromic acid follows a different course in the determination with soil from that in the corresponding blank.

Two molecules of  $\text{CrO}_3$  react with four molecules of  $\text{H}_2\text{O}_2$  in the absence of soil but with three in the presence of soil or ignited soil. The gain in apparent carbon through this error approximately balances the incompleteness of oxidation for the conditions under which Degtjareff appears to have worked.

A new approximate method giving about 76 per cent recovery of carbon is proposed. Finely divided soil is treated with standard potassium dichromate and twice the volume of sulfuric acid added to raise the temperature; after being stirred for a minute the mixture is diluted and the excess dichromate titrated.

This procedure is more rapid than others so far proposed and it is believed that it may prove useful for comparative purposes where no very exact determination is required.

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# A CRITICAL EXAMINATION OF ANALYTICAL METHODS USED IN THE DETERMINATION OF EXCHANGEABLE POTASSIUM AND SODIUM OF SOILS

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Exchangeable potassium and sodium of soils form as a rule not even 10 per cent of the total exchangeable bases, which is an extremely minute quantity, the determination of which is correspondingly difficult. As such, the methods used should be free from errors which have a cumulative effect. The "Proposed Methods" of the Second Commission of the International Society of Soil Science (24), as also those of Kelly and Brown (14) and of Hissink (11), give no details whatever of the determination of the exchangeable alkalies beyond a cursory reference to "usual methods." Russell (18), Schollenberger and Driebelbis (21), Smith (22), and Gedroiz (8, 9) have adopted methods which differ in all details and none of which can claim to be beyond criticism.

Until the Barber and Kolthoff (1) direct method of determining sodium appeared in 1928, and was later extensively tested by Bray (3) and subsequently by Rice Williams (27) with special reference to the determination of exchangeable sodium, the exchangeable alkalies used to be separated and weighed as mixed chlorides or sulfates, in which the potassium was subsequently determined and sodium calculated by difference. The methods of separation were extremely cumbersome and time-consuming, and in the preliminary stages of certain base exchange studies on soils of eastern England (19) involving considerable analytical determinations of the exchangeable alkalies, all the available methods discussed in the following were examined and found to be unsatisfactory, especially for routine work. Of these, Gooch and Eddy's method (10a) of separation, so far not employed in the determination of the exchangeable alkalies, seemed to be the best then available, and was used for certain soils derived from Jurassic and Cretaceous sediments (19). Finally by using ammonium acetate as the replacing agent instead of ammonium chloride, an extremely simple technique adapted for routine determinations was developed for the direct determination of exchangeable sodium by the

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Barber and Kolthoff method (1), and of potassium by the volumetric-cobaltinitrite method, with an immense saving of time and gain in accuracy.

#### METHODS USED FOR THE DETERMINATION OF EXCHANGEABLE SODIUM AND POTASSIUM

*Russell's method.* The method suggested by Russell (18) is most unsatisfactory on both theoretical and practical grounds. After the separation of calcium, sodium is determined by weighing the mixed chlorides or sulfates of magnesium, potassium, and sodium, and subtracting the values found separately for the first two. The sources of error are: (a) Magnesium cannot be separated as an anhydrous chloride, as it is well known that on evaporation on the water bath it forms basic magnesium chloride,  $\text{Mg}(\text{OH})\text{Cl}$ , which on heating forms an oxychloride  $\text{Mg}_2\text{OCl}_2$ , decomposing easily into the oxide  $\text{MgO}$  (14b). Even as sulfate the same difficulties occur;  $\text{MgSO}_4$  containing seven molecules of water and being a salt dehydrates only at  $200^\circ\text{C}$ ., beyond which it tends to decompose into the oxide; (b) Sodium is here estimated by subtracting the values of two quantities both occurring in minute amounts, errors in the determinations of which will make the result for sodium all the less trustworthy: and sodium is an element which requires the most exact evaluation in view of the rôle it plays in the physical and chemical properties of soils.

*Schollenberger and Driebebis* (20) adopt quite a different and tedious procedure. After the separation of calcium by oxalate and the magnesium by ammonium arsenate, the alkalies are determined by evaporating the residue to dryness and converting to sulfates, and expelling the ammonium salts and arsenic. The residual sulfates of the alkalies are filtered off from any insoluble matter (mainly silica) into a 100-cc. Pyrex beaker, evaporated, ignited, and weighed. The potassium is precipitated as  $\text{K}_2\text{PtCl}_6$ , excess plantinic chloride is dissolved in 80 per cent alcohol in a Gooch crucible, the sulfates are removed with half-saturated ammonium chloride, and the latter is removed by final washing with alcohol. From the amount of potassium the sodium is calculated by difference.

This method, with further details and numerous corrections omitted in the foregoing description, is too cumbersome and is even more time-consuming than the method of Gedroiz which is described later. Needless to say, the sodium determination is loaded with all the errors of the analysis, and its accuracy is dependent upon the most thorough separations, and more than all upon the use of extremely pure reagents used only in minimum amounts. The filtrate is bound to be contaminated with sodium salts all through the different stages, in amounts that may have a cumulative effect that will seriously affect the results. It is difficult to conceive how a blank could be carried out under identical conditions, using all the reagents in corresponding amounts.

*Gedroiz's method* (8, 9), followed by A. M. Smith (22) in his study of the

exchangeable bases of Scottish soils, and presumably by most workers, has much to be said in its favor, except that it is too long and that for the separation of all metals except the alkalies milk of lime is used, a reagent which one may doubt could be obtained in an extremely pure form totally free from traces of alkalies compared to ammonium carbonate used in Gooch and Eddy's method (see the following section).

Gedroiz removes the excess of ammonium chloride used for leaching by treatment with concentrated nitric acid on the water bath and the nitrates are then converted to chloride by repeated evaporation with concentrated hydrochloric acid. The alkali chlorides are separated by precipitation of magnesium, iron, aluminum, and manganese with milk of lime (note that the latter three bases always occur in extracts from acid soils); then the calcium is precipitated in the filtrate by repeated treatment with ammonium carbonate and ammonia, warmed on the water bath, followed by filtration. The last process has to be repeated several times and the final traces of calcium must be removed with boiling ammonium oxalate. The separation is obviously tedious and hardly adapted for routine work. Smith (22), who uses the perchlorate method for estimating potassium, removed sulfate with baryta at first, and then separated the rest by repeated precipitation with ammonium carbonate. He did not use milk of lime as Gedroiz does. As discussed in the following, it is questionable whether complete precipitation can be brought about by ammonium carbonate in *aqueous* solution. After the separation of the alkalies, the carbonates are converted to chlorides by repeated evaporations with hydrochloric acid, dried at 100°C., and the last traces of ammonium chloride expelled by ignition, the residue is dissolved in water, filtered from any separated silica, and the mixed chlorides are evaporated, dried gently, ignited, and weighed as  $KCl + NaCl$ .

*Gooch and Eddy's method for separation of the alkalies.* This method, though unique in contrast to the laborious methods described and the extremely unreliable method suggested by Russell (18), has not been adopted in the determination of the exchangeable alkalies as shown by the literature. The separation is claimed to be complete and can be carried out in one operation. It was adopted in the earlier stages of the base exchange studies previously mentioned after the details suggested by Husband and Godden (13), and was found by them to be accurate for the determination of the sodium and potassium in feeding stuffs. The method is that of Kramer (14a) for the determination of sodium and potassium in blood after the method worked out by Gooch and Eddy (10a). The modified method suggested by Husband and Godden is meant to avoid errors arising from the incomplete removal of magnesium. This is overcome by evaporating the solution to dryness before precipitation with *alcoholic* ammonium carbonate (Gooch and Eddy's solution). The method depends on the fact that *alcoholic* ammonium carbonate as recommended by Gooch and Eddy brings about complete precipitation in one operation of all the metals except the alkalies.

In applying the method to the separation of the exchangeable alkalis, the ammonium chloride used for leaching was removed by means of concentrated nitric acid and then evaporated with concentrated hydrochloric acid to dehydrate silica and convert nitrates to chlorides. The residue was taken up in water, neutralized with a few drops of strong ammonia, filtered, and evaporated nearly to dryness to form a thick paste. To this when cold 30 to 35 cc. of Gooch and Eddy's solution was added and well stirred with a glass rod. (Gooch and Eddy's solution is prepared by diluting 180 cc. of strong ammonia—SG.88—to 1 liter with water and mixing it with 1 liter of 90 per cent alcohol. In this mixture 250 gm. of pure ammonium carbonate is dissolved by warming. When cold the solution is filtered and is ready for use. It is desirable to store the solution in Pyrex glass bottles, as ammoniacal solutions dissolve appreciable quantities of sodium from the glass.) The dish is covered with a watch glass, allowed to stand 2 hours, preferably overnight, and then filtered through a Whatman No. 42 filter paper into a small beaker. The precipitate is washed

TABLE 1

*Exchangeable sodium determined by (a) Gooch and Eddy's modified method and (b) the direct method of Barber and Kolthoff*

Soil 22: Amphill Clay

HORIZON	MILLIGRAMS EQUIVALENTS PER 100 GM. SOIL	
	(a)	(b)
Top soil.....	0.58	0.063
Subsoil.....	1.64	0.044
1' 6" to 2' 6".....	2.42	0.323
2' 6" to 3' 6".....	2.74	0.396

with Gooch and Eddy's solution, redissolved in a few drops of concentrated hydrochloric acid, neutralized with ammonia, and reprecipitated and washed again. The combined filtrates are evaporated to dryness in a weighed vitreous dish. The residue is dried and the mixed chlorides are converted to sulfates by heating twice with a few cubic centimeters of pure concentrated sulfuric acid, and the excess of acid is removed by gentle ignition on the sand bath. The dry residue is finally heated in the naked flame, and then in the muffle furnace for 10 minutes, and weighed as the mixed sulfates ( $K_2SO_4 + Na_2SO_4$ ). It is preferable to weigh as sulfates, as these are practically non-volatile compared to the chlorides of the alkalis. Further, as the potassium is determined by the volumetric cobaltinifrite permanganate method, sulfates do not interfere. Blank determinations were carried out with the reagents, "B. D. H"-A. R. reagents being used throughout.

Though the foregoing method is the best of the separation methods available, it was infrequently noted that the residue was stained with iron, an observation confirmed by Blenkinsop (2). The doubt as to the absolute efficiency of the

procedure is always present, and the gravimetric data finally obtained for sodium, especially when these are in the order of a few milligrams, are none too convincing. This is illustrated by the results of table 1 for exchangeable sodium determined by this method and the direct uranyl-zinc-acetate method of Barber and Kolthoff (1), using a technique described later.

#### METHODS PROPOSED

Finally the methods described in the following were adopted with an immense economy of time and a distinct gain in accuracy and are recommended as being quite reliable.

Ammonium acetate was used as the replacing agent instead of ammonium chloride. This reagent introduced by Schollenberger (20) into base-exchange work has immense practical and theoretical advantages detailed by him (20, 21). Its unique practical advantages with special reference to the determination of exchangeable alkalies are: (a) the ease with which it can be removed on the water bath, in contrast to the very unpleasant procedure of removal of excess ammonium chloride by ignition or by treatment with concentrated nitric acid; (b) it does not tend to creep over the edges of the evaporating dishes as does ammonium chloride; (c) the high degree of purity and the ease with which it can be prepared, and in particular the freedom from even traces of sodium salts, whereas on the other hand even the best grade ammonium chloride used ("B. D. H"-A. R.) was found to be contaminated with considerable amounts of sodium salts, a fact confirmed by Piper (17); (d) the ease with which an aqueous extract of the alkali carbonates can be prepared from the replaced acetates of the bases, as detailed later.

"B. D. H"-A. R. ammonium acetate was used when it was freshly prepared. Blank determinations did not show the slightest traces of sodium, but after it had been kept a while it was noticed that amounts of sodium, appreciable compared to the exchangeable sodium, were dissolved from the glass. Consequently required amounts of fresh solutions were always prepared and a series of determinations quickly carried out.

The blank for potassium was always significant, and in all samples examined the blank had to be taken into consideration.

In the aqueous extracts prepared as detailed in the following, potassium was separately determined in duplicate by the volumetric-cobaltinitrite-permanganate method, and the sodium determined directly in two other duplicates by the uranyl-zinc-acetate method.

#### PREPARATION OF EXTRACT

The solutions for the two separate determinations were prepared by the following simple technique:

The whole of the 500 cc. of ammonium acetate from 25 gm. of soil (these amounts were found by preliminary trials to be sufficient for complete removal of exchangeable potassium) was evaporated on the water bath, when the ammonium acetate was removed by volatiliza-

tion. When the evaporation was complete the residue was transferred into a small porcelain dish, re-evaporated to dryness, the dish dried at 110°C. and then over a Rose burner, and when finally dry gently ignited over a small naked flame, at as low a temperature as possible. Because of the minute amounts of potassium and sodium to be determined the final ignition was not carried out in the muffle furnace as was done by Bray and Willhite (4) and Rice Williams (27) in the application of a similar procedure for the determination of the total exchangeable bases of soils. The organic matter very easily burned off, and the acetates were converted to carbonates. When the residue was cool the potassium and sodium were extracted with hot water: the procedure adopted was to moisten the dry residue with water to form a paste, which was finely triturated with a small pestle, and finally extracted with boiling water. In this process the sodium and potassium carbonates are separated from the insoluble residues of calcium and magnesium carbonates. The residue on the filter paper is well washed and the aqueous filtrates are collected in small beakers, ready for the determination of the potassium and sodium.

This extremely simple technique of preparing the solution, associated with the use of ammonium acetate, ensures an immense saving of time, as the processes of ignition and extraction never took more than 1 hour, during which time a whole series of determination can be carried out. The low temperature of ignition and the ease of volatilization of ammonium acetate prevent the serious errors in exchangeable potassium determinations discussed in the following; the potassium is also in the form of the stable carbonate compared to the easily volatile chloride.

For the sodium determination an aqueous solution containing no more than traces of alkaline earths is obtained, in contrast to the technique of Bray (3) and of Rice Williams (27), who take up the ignited residue in hydrochloric acid, neutralize it with ammonia, and thus obtain a solution containing minute amounts of sodium in the presence of "large excess of calcium and magnesium in the form of chlorides." Though the Barber and Kolthoff method for the determination of sodium works in the presence of calcium and magnesium, it would no doubt be an advantage to have a solution containing as small an amount as possible of these salts in the presence of sodium. Besides there is the certain possibility that traces of sodium are not introduced from the hydrochloric acid and ammonia used in the preparation of the extract by the aforementioned authors.

#### RELIABILITY OF EXCHANGEABLE POTASSIUM FIGURES

The question of the estimation of potassium in soils (and it applies with equal emphasis to exchangeable potassium in soils) has received a good deal of attention during recent years. The most popular methods a few years ago were the perchlorate and the platinic-chloride methods, though both these are susceptible to a complexity of errors unlike the cobaltinitrite method when applied to soil extracts. Dodd (6) drew attention to the several sources of error in both the former methods, and strongly urged the adoption of the volumetric-cobaltinitrite method as the official method. When applied to small quantity work, such as the determination of 5 to 10 mgm. of exchangeable potassium,

in the presence of small quantities of calcium sulfate, which invariably occur in minute but appreciable amounts in the soil extract, a gravimetric method is beset with many difficulties compared to a volumetric method.

In the earlier stages of the work on base exchange studies previously mentioned, the details followed in applying the volumetric-cobaltinitrite permanganate method to the determination of exchangeable potassium were those modified by Morris (16) after the original method of Adie and Wood (a1). The exchangeable potassium was determined in the mixed sulfates separated by Gooch and Eddy's method previously described. Later when Milne (15) published his modification of the method as applied to soil extracts, his technique was adopted.

Milne (15), in applying the method to the determination of exchangeable potassium using ammonium chloride, called attention to the possibility of serious error in the values obtained. Adding known amounts of potassium in the form of potassium chloride to the ammonium chloride extracts of two soils, he was able to recover only 60 to 80 per cent of the added amounts, as shown in table 2, after the excess of ammonium salts had been removed, though with

TABLE 2  
*Recovery of potassium from ammonium chloride extracts*

	DIFFERENT ESTIMATE ON SIMILAR ALIQUOTS OF AN $\text{NH}_4\text{Cl}$ EXTRACT	PER CENT RECOVERY OF ADDED AMOUNTS
Soil C (sandy loam) . . . . .	6.13, 5.56, 5.95 mgm. $\text{K}_2\text{O}$	77, 88, 85
Soil D (chalk soil). . . . .	9.89, 9.17, 9.26, 8.12, mgm. $\text{K}_2\text{O}$	61, 77

citric acid extracts no evidence of serious inaccuracies were observed, 96 to 97 per cent of the added potassium being recovered. Though he does not state the method used for removing the excess of ammonium chloride, Milne suggested that it may be associated with the method of destroying a large quantity of ammonium salts, when an appreciably volatile constituent, such as potassium chloride, is to be estimated in the residue. Presumably he had adopted the method of ignition, and losses of potash are bound to occur where potash exists in the form of chlorides subjected to prolonged ignition or even where the ammonium chloride is removed with concentrated nitric acid followed by subsequent ignition. Rice Williams (26), too, subjects the residues to a prolonged ignition of several hours, after preliminary removal of acetic acid used as the replacing agent by evaporation with hydrochloric acid, or ammonium chloride with concentrated nitric acid and subsequent ignition, before obtaining the aqueous extract for the potassium estimation by Milne's method.

As this was an extremely important point to be critically tested, it was decided to investigate whether such losses occur when ammonium acetate is used as the replacing agent, in view of the theories as to the important rôle of ex-



changeable potassium in plant nutrition held by several workers within recent years, such as Hoagland and Martin (12), Fraps (7), Gedroiz (10), Burd (5), Stewart (23), and Wheeting (25), to mention only a few, which necessitate the accurate estimation of exchangeable potassium.

Nine 25-gm. lots of a heavy Oxford clay subsoil (pH 6.7) were extracted with normal ammonium acetate to 500 cc. each. In three the exchangeable potassium was determined in the aqueous extract prepared as previously described. To three of the remaining ammonium acetate extracts before evaporation and ignition, 25 cc. of a solution of potassium chloride containing 5.069 gm.  $K_2O$  was added and to the remaining three, 50 cc. of the same solution containing double the amount of potassium. After evaporation and ignition as previously described, the potassium in the aqueous extracts was determined by the volumetric-cobaltinitrite-permanganate method using Milne's technique. Table 3, which is self-explanatory, shows that *there is practically*

TABLE 3  
*Exchangeable potassium and the recovery of added potassium from ammonium acetate extracts of an Oxford clay soil*

	K <sub>2</sub> O FOUND	MEAN OF 3 TRIALS	ADDED K <sub>2</sub> O RECOVERED	
	mgm.	mgm. K <sub>2</sub> O	mgm.	per cent
1. Exchangeable potassium.....	5.16 5.18 5.17	5.17	....	....
2. Exchangeable potassium with added KCl = 5.069 mgm. K <sub>2</sub> O.....	9.95 10.17 10.23	10.12	4.95	97.6
3. Exchangeable potassium with added KCl = 10.14 mgm. K <sub>2</sub> O.....	14.97 15.22 15.16	15.12	9.95	98.1

*complete recovery of added potassium and that the method is extremely trustworthy.* As previously suggested this may be also due to the fact that potassium is converted from the acetate to the stable non-volatile carbonate—an observation supported by the fact that Milne obtained 96 to 97 per cent recovery in the case of added potassium determined in citric acid extracts of soils, where the potassium citrate is finally converted to carbonate during ignition.

In using Milne's technique it was found extremely convenient and rapid to filter and wash the precipitate in Jena glass filters (grade of porosity 4, capacity 30 cc.), which are undoubtedly preferable to the Gooch crucible with a disc of filter paper pressed well down at the edges and covered with glass dust as recommended by Milne. A distinct advantage was the fact that these could be repeatedly used with equal efficiency and are easy to clean. The permanganate oxidation was done in 800-cc. Pyrex glass beakers. Excellent duplicates were always obtained, as shown in table 3.

## THE DETERMINATION OF EXCHANGEABLE SODIUM

When the mixed alkali sulfates were separated by Gooch and Eddy's method, as previously described, sodium was found by difference after the estimation of potassium. This was abandoned in the later stages of the work and the sodium was directly determined in the aqueous extract prepared as described before by the direct uranyl zinc acetate gravimetric method of Barber and Kolthoff (1).

As this method has been exhaustively examined by Bray (3) and by Rice Williams (27) as applied to the determination of exchangeable sodium of soils, a further examination was considered unnecessary.

## SUMMARY

Analytical methods used in the determinations of exchangeable potassium and sodium of soils are critically reviewed, and the unsatisfactory nature of the methods mentioned in the literature is emphasized. As these bases occur in minute amounts in the absorption complex compared to the alkaline earth bases, accurate and standardized methods are necessary in view of the important rôle exchangeable sodium and potassium play in soils.

It is shown that the ammonium acetate as a replacing salt for the determination of exchangeable potassium gives very reliable results in contrast to the errors associated with the use of ammonium chlorides as observed by Milne. An extremely simple and rapid technique is described.

The determination of exchangeable sodium by the method of Barber and Kolthoff is recommended.

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# AN ELECTROMETRIC-TITRATION METHOD OF FINDING THE pH VALUE AND LIME REQUIREMENT OF SOILS

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The use of the antimony electrode for determining soil reaction has been advocated by several workers,<sup>1</sup> though so far it has not found much favor with the majority of soil scientists. The antimony electrode is simple in operation, but its uniform response to changes in reaction throughout the working pH range has not been generally or sufficiently appreciated.

The erratic behavior of the antimony electrode is definitely traceable to a poorly polished surface. *Since the successful working of this electrode depends on a perfectly smooth and polished surface, after every determination it should be polished with fine emery powder on cloth; or simply with a clean strip of filter paper.*

The usual electrometric method of finding pH value of solutions requires a potentiometer and a sensitive galvanometer. A good potentiometer is an expensive piece of apparatus requiring careful handling. In this paper a simpler technique is described which does away with the potentiometer and makes use of the galvanometer only.

The principle of the method is well known and has been used in a modified form, in the case of the hydrogen electrode. It depends upon the fact that two antimony rods in solutions having the same pH value develop the same potential and if connected together through a galvanometer no current will be shown to be passing. Thus if two antimony electrodes are dipped, one in a solution of unknown reaction and the other in a buffer solution the pH value of which could be altered gradually by the addition of acid or alkali, and the two are connected together through a galvanometer, as shown in figure 1, then the pH value of the unknown solution could be determined by adding gradually acid or alkali to the buffer solution and tapping the galvanometer each time till the zero point is reached. The pH value of the unknown solution will then be equal to that of the buffer solution and can be read from the titration curve of the latter.

## EXPERIMENTAL

Two points are important for the success of the method outlined in the foregoing, namely, the choice of the buffer solution, the titration curve of which

<sup>1</sup> Puri, A. N. 1932 The use of the antimony electrode for determining soil reaction. *Mem. Punjab Irrigation Res. Inst.* vol. 4 (4).

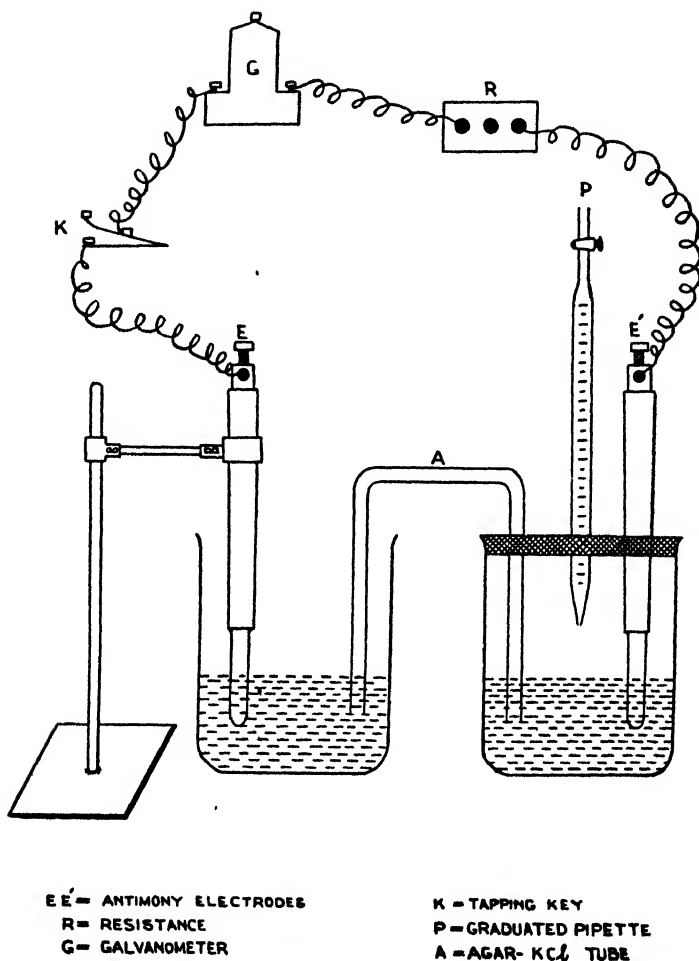


FIG. 1. ARRANGEMENT OF APPARATUS IN THE ELECTROMETRIC-TITRATION METHOD

should cover the whole of the working pH range, and the reproducibility of the antimony electrode potential throughout this range.

The Universal buffer mixture of Prideaux and Ward<sup>2</sup> was chosen for this purpose. It is prepared as follows:

Mix together weighed quantities of the following substances and make up to a liter with freshly boiled distilled water.

1. Potassium dihydrogen phosphate  $\text{KH}_2\text{PO}_4$  = 5.445 gm.
2. Phenyl acetic acid  $\text{C}_6\text{H}_5\text{CH}_2\text{COOH}$  = 5.443 gm.
3. Boric acid  $\text{HBO}_3\text{H}_2\text{O}$  = 2.466 gm.

<sup>2</sup> Prideaux, E. B. R., and Ward, A. T. 1924 Calculations on the neutralisation of mixtures of acids, and a universal buffer mixture. *Jour. Chem. Soc.* 125: 426-429.

The foregoing mixtures can be made in several lots and stored in test tubes or small bottles, well stoppered. The contents of each test tube or bottle only need to be made up to a liter with distilled water when required. The solution keeps well for several weeks. The titration curve of the Universal buffer is given in figure 2.

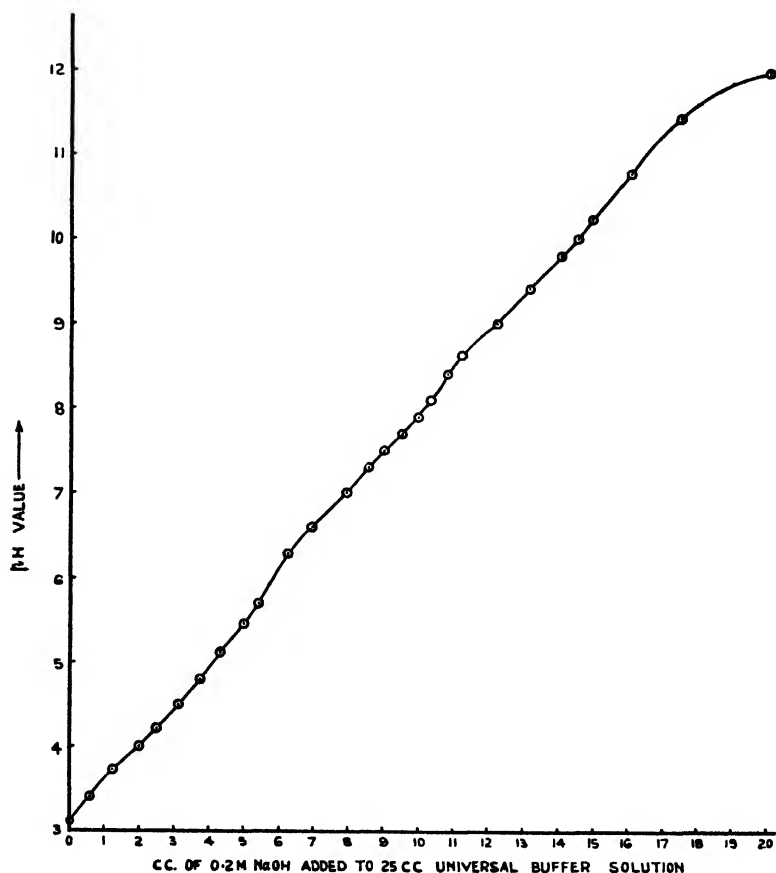


FIG. 2. TITRATION CURVE OF THE UNIVERSAL BUFFER SOLUTION

The electrodes were prepared by melting Merck's pure antimony (Regulus) in a silica dish and pouring it into an iron mould standing vertical and kept heated. The electrodes when cast were about 5 cm. long and about 1 cm. in diameter. They were smoothed and polished on the lathe and soldered on to brass rods having screws at the top for electrical connections. (See fig. 1.)

For the successful working of the method it was essential that the uniformity of the behavior of the antimony electrode throughout the pH range of the Uni-

versal buffer should be tested rigorously. The main item of work in this investigation, therefore, centered round this problem; and the results are important not only from the point of view of this method, but equally so in illustrating the behavior of the antimony electrode in general.

TABLE 1

*Relation between pH and E.M.F. of antimony electrode (KCl — HCl buffer of pH 2.03 used as standard)*

CC. OF 0.2 M NaOH ADDED TO 25 CC UNIVERSAL BUFFER AND VOLUME MADE TO 50 CC	pH	E.M.F. MILLI-VOLTS				
		1	2	3	4	Average
		m.v.	m.v.	m.v.	m.v.	m.v.
0	3.10	54	53	54	53	53.5
0.57	3.40	68	67	68	65	67
1.25	3.73	87	88	93	89	89.25
2.00	4.00	98	...	...	...	...
2.50	4.21	114	115	113	115	114.25
3.10	4.50	129	134	135	135	133.25
3.75	4.80	143	145	146	144	144.50
4.32	5.1	163	167	167	169	166.50
5.00	5.43	198	200	198	198	198.50
5.40	5.70	226	224	224	228	225.50
6.25	6.30	258	257	257	260	258
6.95	6.60	268	269	266	266	267.25
7.90	7.00	287	282	282	287	184.50
8.55	7.30	...	295	292	297	294.67
9.00	7.50	318	312	308	312	312.50
9.50	7.70	336	326	326	327	328.75
10.00	7.91	348	343	342	344	344.25
10.35	8.10	366	...	...	...	...
10.85	8.40	373	367	368	365	368.25
11.25	8.62	379	378	373	378	377
12.25	9.00	392	386	395	386	389.75
13.20	9.40	412	411	415	410	412
14.10	9.80	433	429	434	429	431.25
14.55	10.00	456	452	452	453	453.25
15.00	10.21	474	466	465	469	468.5
16.10	10.75	500	495	500	497	498
17.50	11.41	538	529	532	528	531.75
20.00	11.94	566	560	562	559	561.75

RELATION BETWEEN pH AND E.M.F. OF THE ANTIMONY ELECTRODE, USING  
ANTIMONY ELECTRODE IN KCl — HCl BUFFER OF pH 2.03 AS  
THE STANDARD

A series of buffer solutions ranging from pH 3.1 to 11.94 were prepared from the Universal buffer solution by taking 25 cc. of the latter, adding the required amount of 0.2 M NaOH and making up to 50 cc. The pH values of the buffer solutions were interpolated from a large scale drawing of the titration curve of

the Universal buffer. KCl-HCl buffer of pH 2.03 is easily prepared by mixing 900 cc. of 0.1 *N* KCl with 100 cc. of 0.1 *N* HCl. An antimony rod dipped in this buffer gives an easily reproducible standard electrode in place of the usual calomel half cell.

TABLE 2

*Titration of 25 cc. of Universal buffer against 0.2 M NaOH (Sb in KCl — HCl buffer as standard)*

NaOH ADDED	E.M.F.				
	1	2	3	4	5
cc.	m.v.	m.v.	m.v.	m.v.	m.v.
0	51	49	47	51	59
	> 28	> 29	> 30	> 30	> 31
1	79	78	77	81	90
	> 21	> 22	> 23	> 21	> 20
2	100	100	100	102	110
	> 19	> 22	> 18	> 20	> 20
3	119	122	118	122	130
	> 26	> 24	> 23	> 20	> 20
4	145	146	141	142	150
	> 44	> 44	> 45	> 44	> 44
5	189	190	186	186	194
	> 44	> 44	> 44	> 46	> 44
6	233	234	230	232	238
	> 24	> 23	> 24	> 20	> 22
7	257	257	254	252	260
	> 19	> 19	> 17	> 20	> 18
8	276	276	271	272	278
	> 17	> 17	> 16	> 17	> 19
9	293	293	287	289	297
	> 27	> 27	> 31	> 33	> 33
10	320	320	318	322	330
	> 30	> 28	> 31	> 27	> 27
11	350	348	349	349	357
	> 22	> 23	> 20	> 22	> 22
12	372	371	369	371	379
	> 22	> 19	> 19	> 18	> 18
13	394	390	388	389	397
	> 25	> 25	> 26	> 23	> 23
14	416	415	414	412	420
	> 41	> 31	> 30	> 33	> 23
15	441	446	444	445	453
	> 31	> 36	> 36	> 43	> 43
16	482	482	480	488	496
	> 22	> 31	> 31	> 28	> 28
17	513	513	511	516	524
	> 15	> 21	> 23	> 17	> 17
18	535	534	534	533	541
	> 15	> 16	> 14	> 18	> 18
19	550	550	548	551	559
	> 9	> 7	> 8	> 4	> 4
20	559	557	556	555	563

Measurements of E.M.F. were taken with a Crompton box-type potentiometer using a sensitive mirror galvanometer to find the zero position. Four sets of observations were taken at different times. The results given in table 1 show that the greatest variation is not more than 10 m.v. for the same pH in different observations, corresponding to an average difference of about 0.1 pH.



The electrode was cleaned after every observation. The object of this cleaning is to remove the very thin layer of the oxide that is formed when the rod is dipped in a solution.

In order to determine whether the changes in the E.M.F. of an antimony electrode consequent upon the gradual addition of alkali to the Universal buffer

TABLE 3

*Titration of 25 cc. of Universal buffer against 0.2 M NaOH (quinhydrone in KCl - HCl buffer as standard)*

NaOH ADDED		E.M.F.				
		1	2	3	4	5
cc.	m.v.	m.v.	m.v.	m.v.	m.v.	m.v.
0	500	498	500	490	500	
	>30	>30	>28	>29	>29	
1	530	528	528	519	529	
	>20	>19	>19	>22	>20	
2	550	547	547	541	549	
	>20	>20	>19	>19	>19	
3	570	567	566	560	568	
	>26	>22	>24	>27	>28	
4	596	589	590	587	596	
	>42	>46	>45	>43	>49	
5	638	635	635	630	645	
	>43	>41	>38	>38	>39	
6	681	676	673	668	684	
	>23	>15	>15	>15	>15	
7	704	691	688	683	709	
	>17	>19	>19	>21	>18	
8	721	710	707	704	727	
	>19	>19	>21	>24	>19	
9	740	729	728	728	746	
	>30	>29	>30	>34	>32	
10	770	758	758	762	778	
	>27	>28	>27	>22	>28	
11	797	786	785	784	806	
	>20	>20	>20	>15	>18	
12	817	806	805	799	824	
	>18	>20	>20	>27	>18	
13	835	826	825	826	842	
	>23	>24	>24	>22	>24	
14	858	850	849	848	866	
	>32	>35	>36	>44	>44	
15	890	885	885	892	910	
	>41	>32	>37	>32	>36	
16	931	917	922	924	946	
	>29	>21	>18	>24	>19	
17	960	938	940	948	965	
	>20	>12	>14	>20	>20	
18	980	950	954	968	985	
	>15	>14	>14	>12	>12	
19	995	972	968	980	997	
	>13		> 8	>10	>13	
20	1,008	.....	976	990	1,010	

solution could be reproduced, 25 cc. of the latter were titrated against 0.2 M NaOH. In table 2 are given the various sets of values for the E.M.F. corresponding to successive additions of alkali.

Table 2 shows that titrations done at different times are reasonably reproducible. It is also seen that any change in the initial value of E.M.F. between KCl-HCl and Universal buffer is reflected in the subsequent readings so that the successive increments in the E.M.F., for every addition of 1 cc. of 0.2 *M* alkali, are practically constant in the different titrations. These are also included in table 2.

TABLE 4

*Titration of 25 cc. of Universal buffer against 0.2 M NaOH (antimony electrode in Universal buffer of pH 3.1 as standard)*

NaOH ADDED	E. M. F.		
	1	2	3
cc.	m.v.	m.v.	m.v.
0	6	0	0
	>28	>28	>28
1	34	28	28
	>20	>21	>21
2	54	49	49
	>22	>19	>19
3	72	68	68
	>24	>24	>24
4	96	92	92
	>42	>48	>48
5	138	140	140
	>40		>38
6	178	.....	178
	>18		>18
7	196	.....	196
	>18		>18
8	214	.....	214
	>21		>21
9	235	.....	235
	>28		>28
10	263	.....	263
	>29		>27
11	292	.....	290
	>21		>22
12	313	.....	312
	>19		>18
13	332	.....	330
	>23		>24
14	355	.....	354
	>40		>40
15	395	.....	394
	>36		>36
16	431	.....	430
	>22		>20
17	453	.....	450
	>16		>20
18	469	.....	470
	>13		> 8
19	482	.....	478
	> 7		> 6
20	489	.....	484

## QUINHYDRONE STANDARD ELECTRODE

Quinhydrone electrode in KCl-HCl buffer has been successfully used as a standard by several workers. This electrode was also employed in the titra-

tion of the Universal buffer in conjunction with the antimony electrode. The results given in table 3 show slightly greater variations than in the case of standard antimony electrode in KCl-HCl buffer (c.f. table 2). However the successive increments of E.M.F. on the addition of alkali, again show a satisfactory agreement.

Since the basic material in all these titrations is the Universal buffer, it seemed logical to use this very solution as the standard for reference; and thus simplify matters. The result of such titrations are given in table 4 and show a good agreement.

TABLE 5

*Change in E.M.F. on successive additions of 1 cc. of 0.2 M NaOH to 25 cc. Universal buffer*

ALKALI ADDITIONS	CHANGE IN E.M.F. ANTIMONY IN KCl-HCl	MEAN VALUE MILLI-VOLTS QUINHYDRONE IN KCl-HCl	ANTIMONY IN UNIVERSAL BUFFER
cc.	m.v.		m.v.
0-1	30	29	28
1-2	21	20	20
2-3	20	19	19
3-4	22	25	24
4-5	44	45	45
5-6	44	40	39
6-7	22	20	18
7-8	19	18	18
8-9	17	20	21
9-10	30	30	28
10-11	29	28	28
11-12	22	20	22
12-13	19	19	18
13-14	24	24	24
14-15	30	34	40
15-16	40	38	36
16-17	30	22	21
17-18	20	17	18
18-19	16	14	11
19-20	7	10	6

It was also found that readings with the Universal buffer as standard were much more satisfactory than with quinhydrone or antimony electrode in KCl-HCl buffer. However, the corresponding increments of E.M.F. on the successive additions of NaOH to the Universal buffer are practically the same in the various sets of observations. This is illustrated in table 5, which records the mean of all increments on the successive additions of alkali in the different sets of observations.

#### pH VALUE OF SOILS

The electrometric titration method would commend itself for soil work on account of its simplicity and cheapness. The following is a detailed description of the method.

Buffer solutions are prepared by adding 5, 10, 15, and 20 cc. of 0.2 *M* NaOH to 25 cc. of the Universal buffer. These are used to locate the pH range within which the unknown soil suspension may lie. One antimony electrode is dipped in the soil suspension and the other in the aforementioned buffers one at a time. The suspension and the buffer solution are connected with an agar-KCl bridge, and the two electrodes are joined to a galvanometer through a tapping key and suitable resistances to vary its sensitivity (fig. 1). The direction of the current when the circuit is closed shows whether the soil has a higher or lower pH than the standard. After the buffers between which the true pH value lies have been located, 0.2 *M* NaOH is added to 25 cc. of the Universal buffer in amount equal to that corresponding to the buffer having the lower pH value. The antimony rod is then dipped in it and connected to the one in the soil suspension; 0.2 *M* NaOH is then added slowly through a graduated

TABLE 6  
*pH values of soils as determined by the potentiometric and electrometric titration methods*

SOIL LABORATORY NUMBER	POTENTIOMETRIC METHOD	TITRATION METHOD
	<i>pH</i>	<i>pH</i>
P.C. 9	5.46	5 44
P.C. 6	5 48	5 44
P.C. 12	5 84	5 70
P.C. 3	7 40	7 46
P.C. 1	7 80	7 84
P.C. 2	7 96	8 10
P.C. 4	8 10	8 10
P.C. 11	8 20	8 16
P.C. 5	8.45	8 56
P.C. 8	8 19	8 21
P.C. 10	8 76	8 70
P.C. 7	9 75	9.82

dropping pipette held in the stopper of the beaker containing the buffer solution. The solution is shaken with a circular motion after each addition and the galvanometer deflection noted till the zero point is reached. Resistance is kept in at first and taken out toward the end of titration. As only about 5 cc. of alkali is to be added at the most, the titrations can be made very rapidly. When the balance is attained the pH value of the soil suspension is equal to that of the buffer. From the total number of cubic centimeters added the pH value of the buffer is ascertained from the graph (fig. 2). Each determination takes about 5 minutes. A dozen soils were examined. The results given in table 6 show a good agreement between the potentiometer and the titration method.

#### LIME REQUIREMENTS OF SOILS

The electrometric titrations method is particularly suited for bringing a certain solution to a definite pH value. Lime requirement measurements

which aim at bringing the soil to a known pH value by the addition of lime can be carried out very easily as follows:

A solution of pH 6.5 or 7 or any other desired pH is prepared from the Universal buffer by the addition of an appropriate amount of alkali (*see* fig. 2). One antimony electrode is dipped in this buffer and connected to another in the soil suspension as shown in figure 1. Standard lime or baryta solution is then gradually added to the suspension till the galvanometer shows no deflection. The soil has then attained the desired pH and the lime requirement is calculated from the amount of lime or baryta used for the purpose. In order to determine whether the results are reproducible an acid soil of pH 5.44 was titrated to pH 7 with saturated lime water. Soil in lots of 5, 10, and 15 gm. was taken and required 1.7, 3.7, and 5.3 cc. of lime water respectively. It is not intended to discuss the merits of lime requirement methods, but it might be pointed out that the electrometric titration method is independent of the quantity of soil used. It is well known that a number of methods give different results when the quantity of soil is changed.

#### SUMMARY

A simple electrometric titration method of determining pH value and lime requirement of soil is described.

It makes use of two antimony electrodes, one dipped in the soil suspension and the other in a Universal buffer solution, the pH value of which is continuously varied till a galvanometer through which the two electrodes are joined together shows no deflection. The whole operation of finding pH values is thus reduced to a simple titration.

Lime requirement methods that aim at bringing the soil to a definite pH value can be carried out easily by the technique described in the paper.

## A NEW APPARATUS FOR MEASURING SOIL SHRINKAGE

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Soil shrinkage is an important factor in the behaviour of agricultural soils. The appearance of cracks and their consequent injury to the root system of plants are intimately related to this property of soil colloids.

Haines<sup>1</sup> in his study of the volume change associated with variations of water content in soil has described a method of volume measurement by displacement of mercury in a constant-volume bottle. "This bottle has a ground in perforated stopper like an ordinary specific gravity bottle, and the bottom is closed by a glass disc held by a washer and a brass screw-on cap exactly like the end of a polarimeter tube. The removal of this disc allowed the insertion or withdrawal of the soil prism." The technique consisted simply in weighing the mercury required to fill the bottle with and without the soil prism and calculating the displaced volume.

The present investigation was originally undertaken to study the effect of replaceable ions on soil shrinkage. However, it appeared in the early stages of the work that Haines' constant-volume bottle presented certain difficulties which required very careful manipulation to overcome. The temperature changes due to unavoidable handling can be remedied by having a double wall like that in Dewar flasks, but mercury is apt to get into the brass threads and can prove very troublesome. The apparatus described as follows was found to be very satisfactory for such studies and might prove useful to other workers.

### DESCRIPTION OF THE APPARATUS

The apparatus shown diagrammatically in figure 1 consists of a U-tube with limbs of unequal diameter (31 mm. and 13 mm.). The narrower limb extends to a 100-cc. burette tube attached to it with rubber tubing. It has also a side tube connected to two water reservoirs through pinch cocks, so that the water level in the burette tube can be raised or lowered by opening the upper or lower pinch cock. The U-tube is filled with pure clean mercury, and water is admitted through the side tube until the burette is filled to the top division.

The wider limb of the U-tube has a thick brass cap (also shown separately on a larger scale in the diagram) that fits evenly and has a hole on one side through which passes a thin steel rod ending in a prong. Attached to the

<sup>1</sup>Haines, W. B. 1923. The volume-change associated with variations of water content in soils. *Jour. Agr. Sci.* 13: 296-310.

steel rod is a fine pointed needle. The steel rod can be raised or lowered and clamped in position by the help of a thumb screw. The needle point is viewed through a reading microscope and its point of contact with the mercury surface can be gauged very accurately, as the needle and its shadow can be focussed simultaneously.

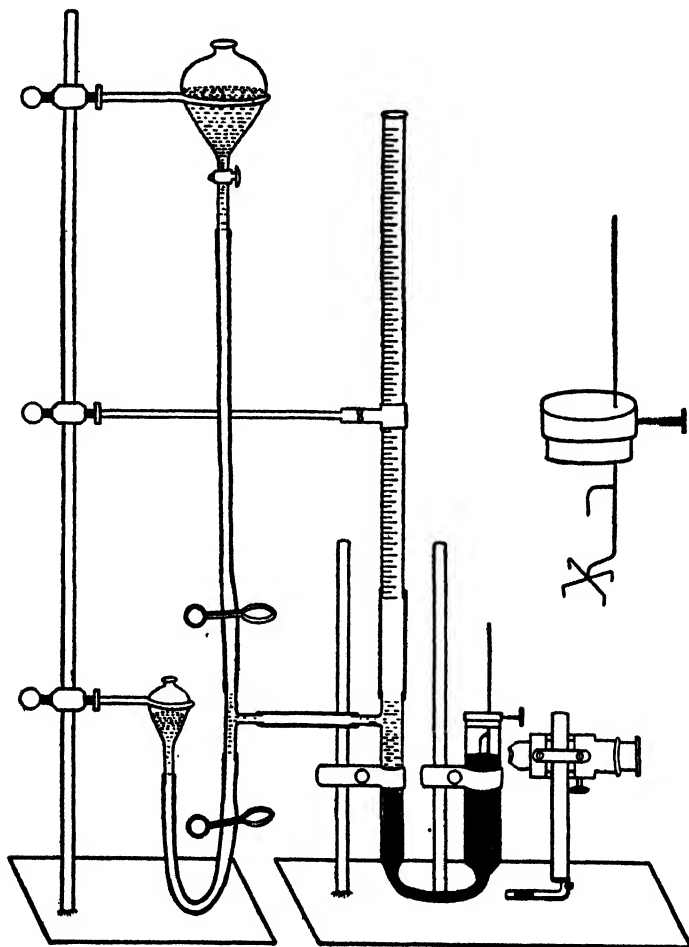


FIG. 1. DIAGRAM OF SHRINKAGE MEASURING APPARATUS

Inset: Brass cap for the wider limb of the U-tube

The zero reading of the apparatus is taken when the needle point just touches the mercury surface and the water level in the burette tube stands near the top. The brass cap is then taken off and soil in the form of a ball is placed on the mercury surface and pushed down with the prong of the steel rod when the cap is replaced in the same position. As a result of the added soil volume, the mercury surface rises. The water level in the burette tube is then gradually

brought down, by opening the lower pinch cock, till the contact is again just made. The fall in the water level is then proportional to the volume of the soil ball, and once the apparatus is calibrated the readings can be taken in a very short time.

Calibration of the instrument is carried out by adding a known weight of mercury to the U-tube, after taking the zero reading, and noting the change in the level of the water level produced thereby. The desired magnification is produced firstly because the density of mercury is higher than that of water, and secondly because the diameter of the limb attached to the burette tube is smaller than the other. To produce a certain change in the level of mercury in the wider limb the level of mercury in the other limb has to be raised or lowered by an amount proportional to the square of the radii of the two limbs.

As the magnification of the instrument can be varied by changing the ratio of the diameters of the two limbs of the U-tube, its sensitivity can be altered to suit individual requirements. In our instrument a magnification of 213 times was attained, so that a change of level of mercury equal to 1 mm. was shown as 21.3 cm. in the burette tube. It might be mentioned that a change in temperature of 5°C. produces a change of less than 0.1 per cent in the magnification due to the alteration in the density of mercury. The calibration, therefore, can be taken to be independent of temperature.

An apparatus of high sensitivity can have only a short total range, for it is not convenient to have a burette tube longer than 100 cm., and consequently it is not possible to measure volumes larger than 3 to 4 cc. For shrinkage experiments this is a decided disadvantage, as volume changes are comparatively small when the total volume has to be kept within 3 to 4 cc. This defect is remedied by taking the zero reading with a glass ball of pre-determined volume (4 to 5 cc.). This volume is added to the soil volume when readings with the latter are taken. The total volume changes due to shrinkage are then comparatively greater, since the soil volume is larger.

#### EXPERIMENTAL

A 30 to 40-gm. portion of air-dry soil, passing through a 1-mm. mesh sieve, is worked into a paste that just does not stick to the hand, a rubber bung at the end of a glass rod being used to break the crumbs and to get it into a uniform plastic condition. It is then shaped into a ball of convenient size. For this purpose a brass disc with a hole is used as a gauge and the ball is made of a size that just slips through. This is essential when working with a number of soils at the same time in order to keep the readings with the different soils within a narrow range of the instrument. .

The soil is worked into a ball, for not only is this shape more convenient to handle but it shows the least tendency to crack on drying. The ball is left in the air for a little while until it is firm enough to be handled without breaking. Its weight is then determined by putting it first into a squat weighing bottle. The weighed soil ball is pushed under mercury in the U-tube and its volume



determined. It is then kept over concentrated sulfuric acid for 2 to 3 hours, after which it is weighed and its volume determined again. In this way a number of readings of weight and volume are taken. The next to the last reading is taken by keeping the soil ball in a vacuum desiccator for 24 hours. The final reading may be taken after the soil has been dried at 100–105°C. in a hot air oven, but it is best to avoid heating.

The results of shrinkage measurements on four soils are shown graphically in figure 2. These have been chosen from a number of soils with a view to

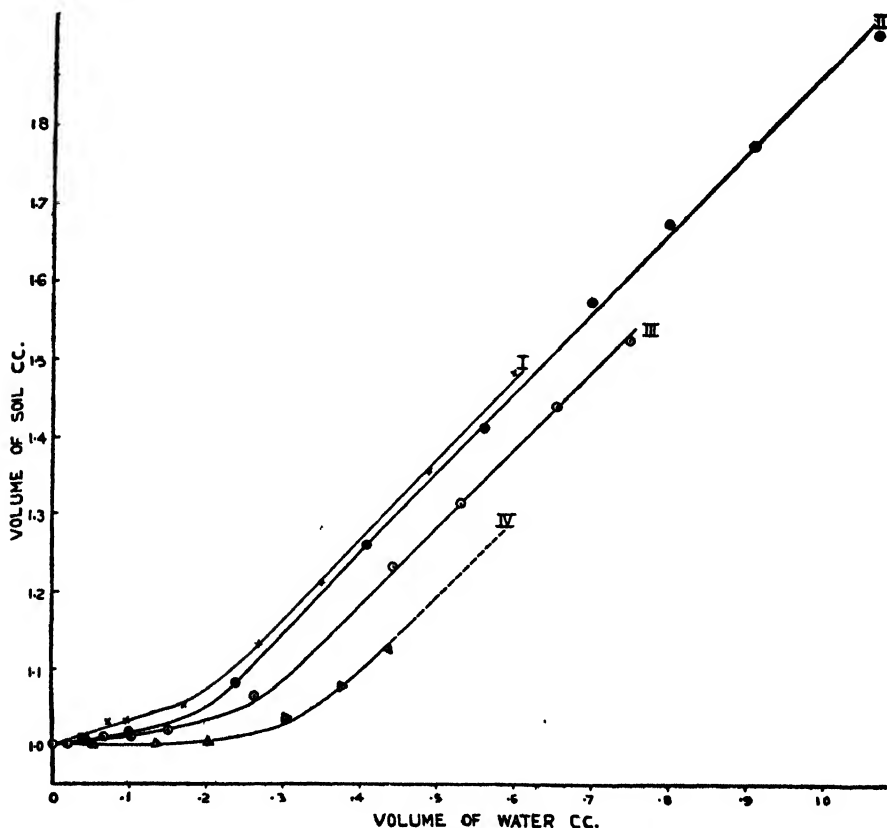


FIG. 2. TYPICAL SHRINKAGE CURVES OF SOILS

convenience of plotting on one scale. The curves are similar to those obtained by Haines, being made up of two portions. The first, or upper, portion of the curve will depend upon the capacity of the soil to imbibe water, and is very short, or even absent, in sandy soils and may be very long in clays. The second, or the lower, portion of the curve depends upon the pore space, since it represents the amount of water lost when the particles have acquired the closest packing. It will be seen that in some soils there is a certain amount of shrinkage along this portion of the curve. Haines has called it "residual shrinkage"

and ascribed it to the films of colloidal material round the solid particles which form small pads between the particles when the first stage of shrinkage has brought them into contact.

The importance of exchangeable bases in modifying physico-chemical properties of soils is well known. An attempt was made to study shrinkage curves of soils saturated with Na, K, Ca, and Ba, in order to determine whether they showed characteristic differences depending upon the nature of the exchangeable bases in them. Soils for this purpose were prepared by leaching with neutral salts, as well as by treatment with hydroxides after removal of the original bases by leaching with 0.05 *N* HCl. The results showed no characteristic differences due to various ions, so that whatever effect the exchangeable ions might have in modifying other physical properties of soils, they have no appreciable influence on shrinkage.

#### SUMMARY

A new apparatus for measuring pore space, apparent density, and shrinkage of moist soil on drying is described.

Typical shrinkage curves of a few soils are given.

The nature of exchangeable ions in soil has no appreciable influence on the character of its shrinkage curve.



# OXIDATION REDUCTION POTENTIALS IN SOILS: I. PRINCIPLES AND ELECTROMETRIC DETERMINATION<sup>1</sup>

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Oxidation reduction potentials of biological systems have been studied by a continuously increasing number of investigators since the suggestive data procured by Gillespie (8) was published in 1920. Warburg (19) has demonstrated the essential character of iron in biological oxidations and Michaelis and Smythe (15) have shown that many iron compounds present in biological systems have definite oxidation reduction potentials. Hopkins (12) pointed out the importance of glutathione in certain biological systems, and Lund (14) showed that it has a stable oxidation reduction potential. The actions of numerous other compounds important in biological oxidations have been shown to be accurately accounted for by their oxidation reduction potentials.

Willis (21) found that liming certain soils to pH 6.2 caused a chlorotic condition of the plants due to the soil manganese becoming unavailable. This can not be explained on the basis of reaction alone, but, as Willis points out, is explainable when the Eh<sup>3</sup> as well as pH of the soil is considered.

The important findings in the field of bacteriology and biochemistry made possible by use of oxidation reduction potentials have made it desirable to obtain more data concerning these potentials in soils. By the use of such data it may be possible to determine the more exact nature of certain soil processes and phenomena which may be important in plant growth. Many phases of the soil-soil microorganism and plant balance in nature may be further explained by the application of similar data in the future.

A search through the literature revealed no rapid, accurate method of determining the oxidation reduction potential of soils while they were in a condition similar to that existing in the field.

This paper reports a comparison of over 50 methods and the proposal of a rapid procedure of sufficient accuracy for comparative tests.

<sup>1</sup> Experimental work reported in this paper was performed in the agronomy laboratories with aid of equipment lent by the department of agricultural and biological chemistry at Pennsylvania State College, State College, Pa.

The author is indebted to Dr. F. G. Merkle, associate professor of soil technology, agronomy department, and Dr. M. W. Lisse, professor of biophysical chemistry, department of agriculture and biological chemistry, for their invaluable aid in preparation of this paper.

<sup>2</sup> Graduate assistant in soil technology.

<sup>3</sup> Eh and oxidation reduction potential are used synonymously in this paper.

The theoretical development of the expression that equilibrates an oxidation reduction potential and the system it represents is given in many theoretical chemistry books (3, 7, 9) and in the excellent works of Clark (4) and Hewitt (11). It will be necessary, however, to repeat a few facts stated in these references in order to make it clear to the soil scientist that this rather new phase of soil study has a precise theoretical foundation.

Beginning with the definition that oxidation is the loss of electrons and reduction is the gain of electrons, Hewitt (11) derives the relation.

$$E_h = E_o - \frac{RT}{nF} \ln \frac{(\text{Ox.})}{(\text{Red.})}$$

where  $E_h$  = the potential in volts, referred to the normal hydrogen electrode as zero, of the system under consideration

$E_o$  = a constant characteristic of the system

$R$  = gas constant = 8.3107 volt coulombs

$T$  = absolute temperature

$F$  = Faraday = 96,500 coulombs

$n$  = number of electrons involved in the change from the oxidized to reduced form

$\ln$  = natural logarithm

(Ox.) = Concentration of oxidized form

(Red.) = Concentration of reduced form.

Since  $R$  and  $F$  are constant,  $T$  may be kept constant,  $n$  assumed equal to 2, and changing to common logarithms the equation becomes:

$$E_h = E_o - 0.03 \log \frac{(\text{Ox.})}{(\text{Red.})} \text{ at } 30^\circ\text{C.}$$

The equation shows  $E_h$  to be dependent on the ratio of oxidant to reductant as well as on temperature and the particular system under consideration. Also the system must be reversible, i.e., the oxidized form readily changing to the reduced form under the influence of suitable  $E_h$  and the reduced form readily changing to the oxidized form. In biological systems the reversibility and proportions of different substances are never fully known. Conant (5), however, points out that even if some compounds present are irreversible, there will be a certain equilibrium present which is accurately described by the oxidation reduction potential revealed by the entire system.

#### ANALOGY TO pH

In describing action or energy, two factors or measurements of the change may be considered; first, quantity factor; and second, intensity factor. The term  $E_h$  relates strictly to intensity and gives no measure of the capacity of the system for oxidation reduction processes.

The following shows the likeness of acidity or basicity to oxidation reduction:

	ACIDITY OR BASICITY	OXIDATION REDUCTION
Measure of quantity factor . . . . .	Titration	Measure of CO <sub>2</sub> evolved
Measure of intensity factor. . . . .	pH	Eh
Term applied to resistance to change of intensity factor . . .	Buffer capacity	Poise

## METHODS OF MEASURING OXIDATION REDUCTION POTENTIALS

Methods of measuring Eh, like those of measuring pH, are of two types; colorimetric, and electrometric.

The dependability of colorimetric methods is not agreed upon. Thornton (18) obtains good results with methylene blue in milk systems, as do Needham and Needham (16) with various indicators in cells. Clark (4) however, reports that methylene blue, a favorite for this type of study, does not react as a reliable reduction indicator. Many European investigators have used indicators in biochemical research, but apparently they are not as reliable for measuring Eh as the indicators used in pH work are for their purpose.

Electrometric methods of measuring Eh show more promise of providing a precise measurement of oxidation reduction intensities than do colorimetric methods. The electrometric method consists of building an electric cell in which the system to be tested is one half and the other half is usually a calomel cell. The unknown half cell consists of an unattackable electrode immersed in the system to be tested. The electrode is connected to the potentiometer and the test solution connected to the calomel cell by means of an electrolyte bridge.

The dependability of numerous types of electrodes for measuring Eh has been tested (2, 4, 6). The common conclusion of these tests is that bright platinum is the most suitable electrode.

Clark and Cohen (4) use a Leeds and Northrup type K potentiometer and a Leeds and Northrup Type R galvanometer for measuring Eh. Hewitt (11) prefers a Lindemann galvanometer with which he notices no errors due to polarization in weakly poised biological systems. Allyn and Baldwin (1) find a vacuum tube potentiometer gives the most sensitive results with bacterial suspensions.

## ELECTROMETRIC METHODS USED IN SOILS

Gillespie (8) measured oxidation reduction potentials of water-logged soils by allowing platinum electrodes to remain in the soil covered with water and measuring the potential each day. The small part of the soil contaminated by the KCl bridge was discarded after each measurement.

Remesow (17) prepared soil suspensions and after allowing them to set for

15 to 18 hours, inserted three electrodes into each and allowed them to stand over night. He then measured the potential at each electrode.

Herzner (10) compared numerous methods of measuring the Eh of soils. He obtained the best results with the soil placed in a Gooch crucible and set in a pan containing a shallow depth of boiled distilled water. When the water appeared at the surface of the soil an electrode of a bright platinum coil of wire sealed in a glass tube was placed in the soil and the potential determined within an hour.

Willis (20) shakes the suspension of soil and water for 3 days in an open flask. Then he transfers the soil suspension to a special electrode flask. The oxygen of the suspension is replaced by purified nitrogen while the system is under reduced pressure. The potential of the oxygen-free soil is then measured.

#### EXPERIMENTAL

The object of the experimental procedure was to find the most accurate method, as indicated by likeness of replicates, that included the following desirable features.

1. Soil as near as possible to the condition it exists in the field. In this connection the treatment of the soil must be such that anaerobic bacteria do not have time to become very active. Also the ratio of soil to water must be as narrow as possible and still insure good contact with the electrode.
2. Determinations must consume as little of the analyst's time as possible.
3. Cost of equipment must be small.

*Preparation of electrodes.* Herzner (10) and Clark (4) found that slight variations in electrodes may not show differences in the potential of highly poised oxidation reduction systems, but may cause decided differences in slightly poised systems like soil or bacterial suspensions. With this in mind electrodes were made of  $3\frac{1}{2}$ -inch lengths of 22 B & S gauge platinum wire. One-eighth of an inch of the wire was fused to a 6-inch length of 18 B & S gauge copper wire. The copper wire was drawn through a  $4\frac{1}{2}$ -inch length of small bore glass tubing and sealed into position as shown in figure 1.

The hook of copper wire was placed in a small well of mercury to make contact with a wire to the potentiometer when the potential of the electrode was being measured.

Twelve electrodes showed the same potential within 0.5 millivolt for a 0.01 *M* potassium ferrocyanide, 0.001 *M* potassium ferricyanide, and 2 *M* KCl solution.

*Soils used.* Hagerstown clay loam surface, Dekalb loam surface, and Moshannon clay loam surface soils were used. They all gave about the same variation for a single method, and only the results for the Moshannon soil are here reported. The soils had all been stored in bins in the air-dry condition for from 1 to 2 years when the potentials were determined.

*Potentiometer and galvanometer.* A Leeds and Northrup type K potentiometer and a Leeds and Northrup enclosed lamp and scale galvanometer were used. The galvanometer has a sensitivity of 40 megohms, a coil resistance of

970 ohms, an external critical damping resistance of 17,000 ohms, and a period of 3.2 seconds. Although a more sensitive galvanometer probably could be used to advantage, no polarization effects were noticed after the first potential for each soil was read, and only occasionally did these effects bother in the first reading. A very quick light tap of the contact key to the galvanometer was practiced in order to guard against polarization. A saturated calomel cell was used as a reference electrode.

*Procedures.* In most of the methods or procedures vials of 30-cc. capacity and 3 inches long were used as containers for the soil suspensions. In one group of methods, however, it was necessary to use Gooch crucibles to hold the soil and water mixture. All trials were made in quadruplicate. The deviation of the mean was calculated by Bessel's formula (13) for experimental error and used as an indication of the likeness of the four values obtained for a certain method and soil.

The first group of trials was designed to show the effect of increasing lengths of time that soil, water, and electrodes were in contact with one another. In this study a soil to water ratio of 1 to 1 was used. The soil and water were thoroughly pestled together before the electrodes were inserted, and the soil suspension was stirred with the electrode just prior to measuring the potential. Table 1 shows the results of this first study.

Table 1 shows that 12 hours or more of water-logging causes a decided negative shift in the potential of a soil. Soil and water contact of very short or very long periods apparently produces potentials that are very variable. The results in table 1 indicate that the method of Remesow (17), which involves water-logging for at least 24 hours, allows considerable opportunity for a negative shift in potential.

The second set of procedures compared the one recommended by Herzner (10) with modifications of this method. In these tests contact between the soil suspension and electrode was assured by tapping the Gooch crucible and contents on a rubber stopper several times. Methods 10 to 14 in table 2 give the results obtained in this group of tests.

Numbers 10 and 12 showed a low enough deviation of the mean to be promising procedures. However, number 10 shows such a high Eh mean, probably due to placing the electrode in the dry soil, that it was discarded in favor of other methods that gave as uniform results of a lower mean value. Number 10 and especially number 12 showed much less dependable results with other soils than with the Moshannon soil. All of these methods were finally discarded as being less reliable and a little more difficult to manipulate than ones studied later.

The trials numbered 15 to 23 in table 2 were designed to test the effect of

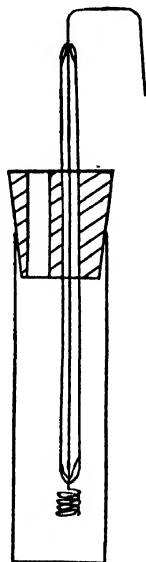


FIG. 1. TYPE OF HALF CELL USED



short-time treatment of the soil suspension with purified nitrogen gas. The very large deviation of the mean with all variations tried shows that a strenuous manipulation of the soil is probably necessary to remove the oxygen uniformly from the soil suspension. The quantitative removal of oxygen from the soil suspension is assumed to be unnecessary and perhaps undesirable, as it creates a condition in the soil quite different from that in the field.

Methods numbered 24 to 29 in table 2 give results obtained when the soil suspension was treated with heat, reduced pressure, and centrifuging.

Numbers 25 and 26 gave much larger deviations of the mean with other soils than with the Moshannon soil. Number 29 was selected for further study and the others were discarded as giving results too variable.

Further studies were decided upon from the results shown in tables 1 and 2, and similar data on other soils. Methods 3, 4, 5, and 29 were selected as more likely to lead to a satisfactory procedure.

TABLE 1  
*Effect of varying lengths of time soil, water, and electrode were in contact*  
Results obtained with Moshannon soil, pH value 5.8

METHOD NUMBER	TIME OF CONTACT	Eh MEAN	DEVIATION OF MEAN
		<i>volts</i>	<i>volts</i>
1	10 min.	+0.529	±0.008
2	30 min.	+0.528	±0.005
3	1 hr.	+0.520	±0.003
4	2 hr.	+0.530	±0.002
5	4 hr.	+0.518	±0.003
6	6 hr.	+0.480	±0.009
7	12 hr.	+0.436	±0.005
8	116 hr.	-0.040	±0.005
9	240 hr.	-0.146	±0.014

The effect of soil to water ratio, time (within rather narrow limits) of soil water and electrode contact, time of centrifuging, pestling soil and water together before inserting electrode, and stirring with electrode just before measuring the Eh were studied. Table 3 shows the results of these studies.

Trial 30 was on a bin sample taken at a date previous to the thoroughly homogenized sample used for all other methods shown in table 3.

Procedures 31 to 36 show that similar results will be obtained if the time of electrode, water, and soil contact is between 1 and 5 hours. They also show that nothing is gained by stirring the suspension with the electrode just prior to making the potential measurements.

Methods 37 to 42 show that soil to water ratios ranging from 7 gm. of soil and 7 cc. of water to 7 gm. of soil and 4 cc. of water give a closer agreement of replicates than ratios in which less water was used in this soil (a clay loam). Numbers 37 to 48 show that a soil, water, and electrode contact of 35 minutes

TABLE 2

*The results of various methods of determining the Eh value of the Moshannon soil  
pH value 5.8*

METHOD NUMBER	SOIL TO WATER RATIO*	TIME OF CONTACT†	TREATMENT	Eh MEAN	DEVI- ATION OF MEAN
				volts	volts
10	Maximum water capacity	90 min.	Electrode and dry soil in Gooch crucible, water from below, and Eh measured after 1½ hours	+0 556	±0 002
11	Maximum water capacity	15 min.	Soil in Gooch crucible, water from below electrode in soil 15 minutes prior to Eh reading	+0 516	±0 005
12	Maximum water capacity	60 min.	Soil in Gooch crucible, water from below and stand one hour, then electrode in soil 1 hour	+0.491	±0 001
13	Maximum water capacity	2 hr.	Soil in Gooch crucible, water from below, and stand one hour, then electrode in soil 2 hours	+0 515	±0 004
14	Maximum water capacity	15 hr.	Soil in Gooch crucible, water from below and stand one hour, then electrode in soil 15 hours	+0 395	±0 010
15	7:7	140 min.	Electrode, soil and water in contact 1 hour under reduced‡ pressure. Released with N <sub>2</sub> stirred, pressure reduced for 1 hour again and released with N <sub>2</sub>	+0 378	±0.019
16	7:7	120 min.	N <sub>2</sub> bubbled through suspension for 10 minutes just before Eh was measured	+0 436	±0.023
17	7:7	135 min.	N <sub>2</sub> bubbled through suspension for 15 minutes just before Eh was measured	+0 491	±0 010
18	7:7	15 min.	N <sub>2</sub> bubbled through suspension for 15 minutes	+0 484	±0.012
19	7:7	13 hr.	Na <sub>2</sub> bubbled through suspension for 10 minutes just before Eh was measured	+0 354	±0 015
20	7:7	15 hr.	Soil, water and electrode in contact under reduced pressure. Released with N <sub>2</sub>	+0 296	±0 027
21	7:7	90 min.	Stirred thoroughly with a stream of N <sub>2</sub> before electrode was inserted and just before Eh was read	+0.455	±0 017
22	7:7	3 hr.	Stirred with N <sub>2</sub> stream before and during Eh measurement	+0.498	±0 008
23	7:7	20½ hr.	Stirred thoroughly with stream of N before electrode was inserted	+0.362	±0 012
24	7:7	2 hr.	Reduced pressure 1 hour, released, suspensions stirred, reduced pressure 1 hour again, released stirred and Eh determined	+0.450	±0.008

\* First figure is soil in grams, second figure water in cubic centimeters.

† Soil, water, and electrode contact.

‡ Reduced pressure in all cases was about 4 cm. of mercury.

TABLE 2—*Concluded*

METHOD NUMBER	SOIL TO WATER RATIO*	TIME OF CONTACT†	TREATMENT	Eh MEAN	DEVIATION OF MEAN
				<i>volts</i>	<i>volts</i>
25	7:7	70 min.	Heated to 90°C., pressure reduced for 40 minutes, released, cooled for 25 minutes, stirred and Eh read	+0.430	±0.004
26	7:7	3 hr. 15 min.	Reduced pressure 3 hours, released, stirred and Eh determined	+0.515	±0.003
27	7:7	14 hr.	Reduced pressure 14 hours, released, stirred and Eh determined	+0.351	±0.021
28	7:7	30 min.	Reduced pressure 20 minutes, released, stirred and Eh determined	+0.513	±0.010
29	7:7	35 min.	Centrifuged for 5 minutes just prior to Eh measurement	+0.466	±0.002

TABLE 3

*Effect of soil: water ratio, centrifuging and other variations on stability of Eh measurement*  
Results with Moshannon soil, pH value 5.8

METHOD NUMBER	SOIL: WATER RATIO*	TOTAL TIME SOIL, ELECTRODE, AND WATER WERE IN CONTACT	TIME CENTRIFUGED	SOIL AND WATER PESTLED AT START	STIRRED WITH ELECTRODE JUST BEFORE MEASURING	VIAL AND CONTENTS TAPPED TO MAKE CONTACT WITH ELECTRODE	Eh MEAN	DEVIATION OF MEAN
							<i>volts</i>	<i>volts</i>
30	5:3	2 hr.	.....	Yes	Yes	No	+0.515	±0.003
31	7:4	75 min.	.....	Yes	Yes	No	+0.461	±0.002
32	7:4	2 hr. 40 min.	.....	Yes	Yes	No	+0.457	±0.003
33	7:4	4 hr. 50 min.	.....	Yes	Yes	No	+0.458	±0.003
34	7:4	1 hr.	.....	Yes	No	No	+0.455	±0.002
35	7:4	2 hr.	.....	Yes	No	No	+0.463	±0.002
36	7:4	3 hr.	.....	Yes	No	No	+0.460	±0.004
37	7:7	35 min.	5 min.	Yes	No	No	+0.466	±0.002
38	7:5	35 min.	5 min.	Yes	No	No	+0.477	±0.002
39	7:4	35 min.	5 min.	Yes	No	No	+0.465	±0.002
40	7:35	35 min.	5 min.	Yes	No	No	+0.464	±0.007
41	7:32	35 min.	5 min.	Yes	No	No	+0.468	±0.005
42	7:3	35 min.	5 min.	Yes	No	No	+0.481	±0.005
43	7:4	65 min.	5 min.	Yes	No	No	+0.445	±0.001
44	7:4	15 min.	5 min.	Yes	No	No	+0.473	±0.004
45	7:4	45 min.	5 min.	Yes	No	No	+0.473	±0.005
46	7:4	50 min.	10 min.	Yes	No	No	+0.479	±0.005
47	7:4	20 min.	10 min.	Yes	No	No	+0.468	±0.003
48	7:4	125 min.	5 min.	Yes	No	No	+0.468	±0.002
49	7:35	35 min.	10 min.	No	No	Yes	+0.455	±0.006
50	7:32	40 min.	10 min.	No	No	Yes	+0.474	±0.004

\* First figure is soil in grams, and second figure is water in cubic centimeters.

TABLE 4

*Eh, mean Eh, deviation of mean, and pH of soils of widely varying properties when determined by the method of pestling and centrifuging*

SOIL	HORIZON	DEPTH	Eh	MEAN Eh	DEVIATION OF MEAN	pH
		<i>inches</i>	<i>volts</i>	<i>volts</i>	<i>volts</i>	
Hagerstown silt loam (a virgin profile from near State College, Pa. Collected by Dr. F. G. Merkle) A typical Gray Brown Forest Soil	A0*	0-1	+0 524			
	A0	0-1	+0.526			
	A0	0-1	+0 539*			
	A0	0-1	+0.529	+0.530	±0.002	4.41
	A1	1-3	+0 533			
	A1	1-3	+0.516			
	A1	1-3	+0 523			
	A1	1-3	+0 524	+0 524	±0.002	4.89
	A2	3-6	+0 517			
	A2	3-6	+0 513			
	A2	3-6	+0 514			
	A2	3-6	+0 518	+0.516	±0.001	4.92
	B1	6-20	+0.505			
	B1	6-26	+0 518			
	B1	6-20	+0 504			
	B1	6-20	+0 510	+0.509	± 0.002	5.29
	B2	20-48	+0 529			
	B2	20-48	+0 530			
	B2	20-48	+0.525			
	B2	20-48	+0 526	+0.528	±0.001	5.13
Otisville Sandy Loam (A virgin profile from near Equinunk, Pa. Collected by L. A. Brown) A typical podsol	A0*	0-3	+0.565			
	A0	0-3	+0 554			
	A0	0-3	+0 566			
	A0	0-3	+0.562	+0.562	±0.002	3.67
	A1	3-5	+0.628			
	A1	3-5	+0.625			
	A1	3-5	+0 636			
	A1	3-5	+0 628	+0 628	±0.002	3.79
	B1	5-6	+0 489			
	B1	5-6	+0 487			
	B1	5-6	+0.496			
	B1	5-6	+0.504	+0.494	±0.002	3.70
	B2	6-13	+0.507			
	B2	6-13	+0.506			
	B2	6-13	+0.496			
	B2	6-13	+0.490	+0.500	±0.003	4.46

\* In these organic layers 3 gm. of material to 10 cc. of water was used to make the test.

TABLE 4—*Concluded*

SOIL	HORIZON	DEPTH	Eh	MEAN Eh	DEVIATION OF MEAN	pH
		<i>inches</i>	<i>volts</i>	<i>volts</i>	<i>volts</i>	
A typical podsol— <i>Concluded</i>	B3	13-23	+0.500			
	B3	13-23	+0.509			
	B3	13-23	+0.517			
	B3	13-23	+0.509	+0.509	±0.002	4.82
	C	23-29	+0.489			
	C	23-29	+0.479			
	C	23-29	+0.484			
	C	23-39	+0.484	+0.484	±0.001	5.47
Matanzas Clay (Perdigan phase from Cuba)	Soil	0-6	+0.466			
	Soil	0-6	+0.468			
	Soil	0-6	+0.470			
	Soil	0-6	+0.465	+0.467	±0.001	7.67
	Subsoil	6-20	+0.485			
	Subsoil	6-20	+0.477			
	Subsoil	6-20	+0.481			
	Subsoil	6-20	+0.483	+0.482	±0.001	7.79

is sufficient for accurate results if the mixture is centrifuged for the last 5 minutes of the period of contact.

In view of the results obtained by use of these 50 methods, number 39 has been selected as best combining simplicity, rapidity, and dependability of results. Procedure 39 was further used on 13 soils to test its dependability on soils differing widely with respect to the nature and quantity of colloidal material present. Results of this test are given in table 4.

All horizons of the Hagerstown soil had been stored in the laboratory, air-dry for 2 years; the Otisville had been stored in similar condition for 6 months; and the Matanzas for 2 months. The method used gives comparative Eh values. However, we must look to future studies for an accurate interpretation of oxidation reduction potentials when they are determined on soils of known physical, chemical, and microörganic properties.

#### DEPENDABILITY OF RESULTS

Bessel's formula, which was used in calculating the deviation of the mean, was further employed to calculate the probable error of the difference of two values. It shows that a difference between means of .010 volt will be a significant difference 40 out of 41 times that two mean values such as those in table 4 are compared.

On the basis of the results reported in table 4 the method used to obtain them is suggested as a reliable, simple method of measuring the oxidation reduction potentials of soils.

## DETAILS OF METHOD

The soil is ground with a rubber pestle to break all granules. Solid particles larger than 1 mm. in diameter are sieved out. The sieved sample is thoroughly mixed. For each measurement 7 gm. of soil in a 30-cc. vial is used. To the soil is added 4 cc. of distilled water. More than 4 cc. of water may be necessary for heavy clay soils. The ratio within narrow limits is not important, however (see table 3, methods 37 to 42). The soil and water are rubbed into intimate contact with a glass rod.

The electrodes are cleaned just prior to each determination either by immersing in hot strong  $\text{HNO}_3$ , rinsing with water, then immersing in 2  $N$   $\text{NH}_4\text{OH}$  and rinsing with distilled water; or by immersing in chromic sulfuric acid cleansing solution a few minutes, rinsing in distilled water, and flaming in an alcohol flame. The latter method gives slightly better results. The electrode is placed in the suspension of soil in water within a few minutes after the suspension is prepared. The electrode is held in its rubber stopper in such a manner that the platinum spiral is completely submerged, but does not touch the bottom of the vial (see fig. 1).

The soil suspension and electrode are left in contact for 30 minutes. The vial and contents are then placed in a centrifuge (the bottom of the centrifuge cups were padded with cotton to prevent breakage of the vials) and centrifuged at 256 times gravity for 5 minutes. The potential of the suspension is measured as soon as the centrifuge is stopped.

## SUMMARY

A few of the principles of oxidation reduction potentials applicable to biological systems are briefly restated from previous publications (4, 11).

A comparison of 70 methods (details of 50 are given) of measuring oxidation reduction potentials revealed a procedure which embodies the following qualities:

Soil very near its condition in the field when potential is measured.

Each determination consumes a comparatively short period of the analyst's time.

Little equipment other than that used in the electrometric measurement of pH is needed.

A method is proposed in which the soil and water mixture is thoroughly pestled, the electrode inserted, and the whole centrifuged in order to insure intimate contact. The Eh is read immediately. Potentials obtained by this method are comparative, and have a deviation of the mean of not more than .003 volt.

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## BOOK REVIEW

*American Peat Deposits.* By A. P. DACHNOWSKI-STOKES and V. AUER. In *Handbuch der Moorkunde*, VII. Gebr. Borntraeger, Berlin, 1933. Pp. vi + 242, pl. 9, figs. 43.

Recent progress in peat investigations is due in large measure to the problems presented by a desirable utilization of "submarginal" land and by the demand for standard grades of commercial peat products. That a fuller understanding of vertical cross sections and a more detailed description of peat profiles have the broadest application in physical and biochemical studies of organic matter, as well as for practical purposes is now conceded. That greater emphasis should be laid on genetic, historical, and developmental features of peat land for purposes of classification has been realized only recently.

The book just issued is volume VII of a series of 10 books contributed by specialists of different countries, and edited by Dr. K. von Bülow.

Part I of the book deals with peat deposits in the United States and their characteristic profiles and classification, and has been written by Dr. A. P. Dachnowski-Stokes of the U. S. Bureau of Chemistry and Soils. The major groups of peat land are associated with predominating environmental conditions of climate, vegetation, and land relief. The subdivisions have been outlined on the basis of inherent morphological and developmental features of distinctive type profiles and on the degree of decomposition and characteristic properties of peat soils produced in different environments.

Part II presents a general and interpretative contribution on "Peat bogs in southeastern Canada" by Dr. V. Auer of Finland. The use of pollen-analysis technique by the author indicates that changes in forests and presumably in climate correspond to those established in Europe.

Part III is entitled "Die Moore Südamerikas, insbesondere Feuerlands" and is also written by Dr. Auer. The graphical presentation of highmoors in Tierra del Fuego and the significant discussion of the changes in vegetation should make this part particularly helpful to the more technically minded student.

The "America" volume deserves closer consideration by workers in pedology, ecology, and geography, and by those whose interest lies in the agricultural, industrial, and other uses of peat land resources.

A. G. McCall.





# THE INFLUENCE OF COPPER SULFATE ON IRON ABSORPTION BY CORN PLANTS<sup>1</sup>

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It is widely known that many peat soils require heavy fertilization with potash. Hoffer<sup>3</sup> has shown very conclusively that at least a part of the efficiency of potash for corn can be ascribed to its influence in preventing an excessive intake of iron and the accumulation of this element in the nodes. Only one rate of application, 480 pounds of 12 per cent kainit, was used in the experiments from which Hoffer got his data and, since other work on upland soils as fully depleted of potash as was the peat indicates that the maximum requirement of corn for potash is only about 30 pounds of  $K_2O$ , there is some evidence that potash on the peat soils has some function other than as a nutrient.

Since Hoffer had no data with lesser amounts of potash and since no other experimental work on the problem is available, some results of a demonstration conducted by J. L. Rea, assistant director of the State Experimental Farm at Wenona, N. C., are used as evidence of the high potash requirements of these soils.

The farm on which this work was done had been highly productive for a period of 10 years after the land was cleared without the addition of any lime or fertilizer. Within 3 years thereafter the yield of corn decreased to negligible amounts on some fields. Liming at a rate of 3 tons per acre did not correct the condition.

The results of the demonstration are given in table 1.

Corn plants on the peats receiving no potash were stunted. Yellow striped leaves forming a compact bunch at the top of the stalk gave evidence of the failure of the latter to elongate. Root rot was severe, and there were heavy accumulations of iron in the nodes. There was a distinct gradation from this condition to fully normal plants as the rate of addition of potash was increased. With such evidence it might be questioned whether or not the effect of potash was specific. Since similar response was evidenced in some experiments with

<sup>1</sup> Contribution from the department of agronomy, North Carolina Agricultural Experiment Station. Publication as paper No. 74 of the Journal series authorized by the director.

<sup>2</sup> Soil chemist and assistant respectively.

<sup>3</sup> Hoffer, G. N. 1926 Testing corn stalks chemically to aid in determining their plant foods needs. Purdue Univ. Agr. Exp. Sta. Bul. 298.

copper sulfate, a pot experiment was planned to determine the influence of this compound on iron absorption. A lot of peat soil was taken from an unproductive spot in a field on the experimental farm at Wenona and put into 4-gallon pots in the greenhouse. Lime was applied at rates of 5 and 10 tons per acre of 1 million pounds. The treatments were mixed throughout the soil. No fertilizer was added. Copper sulfate was added at the rate of 1,000 pounds per acre.

An upland variety of corn was planted, 10 seed to the pot, and the soil was watered with distilled water to maintain a fairly uniform moisture content. Germination was normal and the seedlings were thinned to three plants to the pot. Each treatment was duplicated.

It soon became evident that the plants on the unlimed soil without copper were not making normal growth, and the symptoms were similar to those seen in the field. With copper on the unlimed soil the plants were taller and more thrifty but these also developed the same yellow striped leaves. Both rates of liming were beneficial and produced uniformly healthy green leaves although

TABLE 1  
*Yield of corn on a peat soil with muriate of potash*

RATE OF APPLICATION OF KCl	YIELD PER ACRE
<i>pounds</i>	<i>bushels</i>
None	7
32	22
64	31
128	51

the leaves of the plants on soils not receiving copper sulfate wilted when the temperature of the greenhouse became high.

The appearance of the plants with the different treatments at the time of cutting is shown in figures 1 and 2 of plate 1.

At both rates of liming and with copper sulfate added the plants were slightly better than with the lime alone, and the leaves kept their normal turgor under all conditions.

Just before tasselling the plants were cut and each entire plant was handled as a separate sample. These were dried, weighed, and burned to ash at a low temperature in an electric muffle furnace. Iron was determined in the ash of the whole plant by the Stokes and Cain colorimetric method. The results are given in table 2.

While the plants were fresh the stalks were split. The accumulation of iron in the nodes of the plants grown with the heavy rate of liming is shown in figure 3 of plate 1.

When the plants were cut from the pot it was noted that the roots in the soils receiving lime and copper were exceptionally bright and healthy. This sug-

gests that the copper might act as a fungicide at this heavy rate of application. If this is so it appears to be limited to a few species of the soil organisms, since further work has shown that the organic matter of this soil is more rapidly decomposed with copper and lime than with lime alone.

Ordinarily it would be expected that so much copper would be toxic to plants. It appears to be fixed in so insoluble a form, however, by the organic matter that it does not even affect the accuracy of a hydrogen electrode.

The physiological significance of these data is difficult to interpret with any degree of assurance on account of differences in the size of the plants. From the ash analysis it appears that liming alone has increased the ratio of iron to total ash constituents, whereas the addition of copper with the lime has held the iron:ash ratio nearly constant. The percentage of ash in the plant is, however, inversely related to the amount of dry matter, and when the ratio of iron to dry matter is considered it does not appear that liming alone has

TABLE 2  
*Yield and iron ( $Fe_2O_3$ ) content of corn in peat soils with lime and copper sulfate*

RATE OF LIMING	DRY WEIGHT PER PLANT	ASH CONTENT	IRON CONTENT OF ASH	IRON CONTENT OF PLANTS
<i>tons</i>	<i>gm.</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Without copper sulfate				
None	2.84 $\pm$ 0.07	19.98	0.183 $\pm$ 0.005	0.037
5	23.55 $\pm$ 1.26	9.04	0.419 $\pm$ 0.023	0.038
10	22.36 $\pm$ 1.40	9.74	0.381 $\pm$ 0.013	0.037
With copper sulfate				
None	18.17 $\pm$ 2.00	9.35	0.216 $\pm$ 0.015	0.020
5	31.50 $\pm$ 4.08	7.62	0.314 $\pm$ 0.020	0.024
10	26.90 $\pm$ 1.75	8.79	0.214 $\pm$ 0.007	0.019

influenced the intake of iron although the effect of copper is more pronounced. Probably the higher ash content of the smaller plants is the result, rather than the cause, of the failure of the latter to grow. By any method of comparison, however, the effect of copper is to reduce the intake of iron.

The favorable effect of copper may be due in part to other functions, but in so far as the growth of these plants has been limited by an excessive absorption of iron the increases with the addition of copper can be associated with the control of this factor alone.

The influence of the copper on the intake of iron and the accumulation of this element in the nodes is identical with that of potash salts. These symptoms, therefore, are not specific for potash deficiency, and the effect of potash in controlling them is probably distinct from its function as an essential nutrient. It is also probable that the iron accumulations are due to a peculiar soil condition and that potash deficiency can occur without the development of these symptoms.

## SUMMARY AND CONCLUSIONS

Liming some unproductive acid peat soils does not always bring about favorable conditions for the growth of corn. An unfavorable condition developed by liming is associated with excessive absorption of iron and lodgement of iron in the nodes.

Heavy potash applications or the addition of copper sulfate corrects this condition. The symptom of iron accumulation in the nodes of corn is, therefore, not specific for potash deficiency.

This effect of potash is not necessarily evidence of a nutrient deficiency, nor is the beneficial influence of copper due entirely to the function of this element as a nutrient.

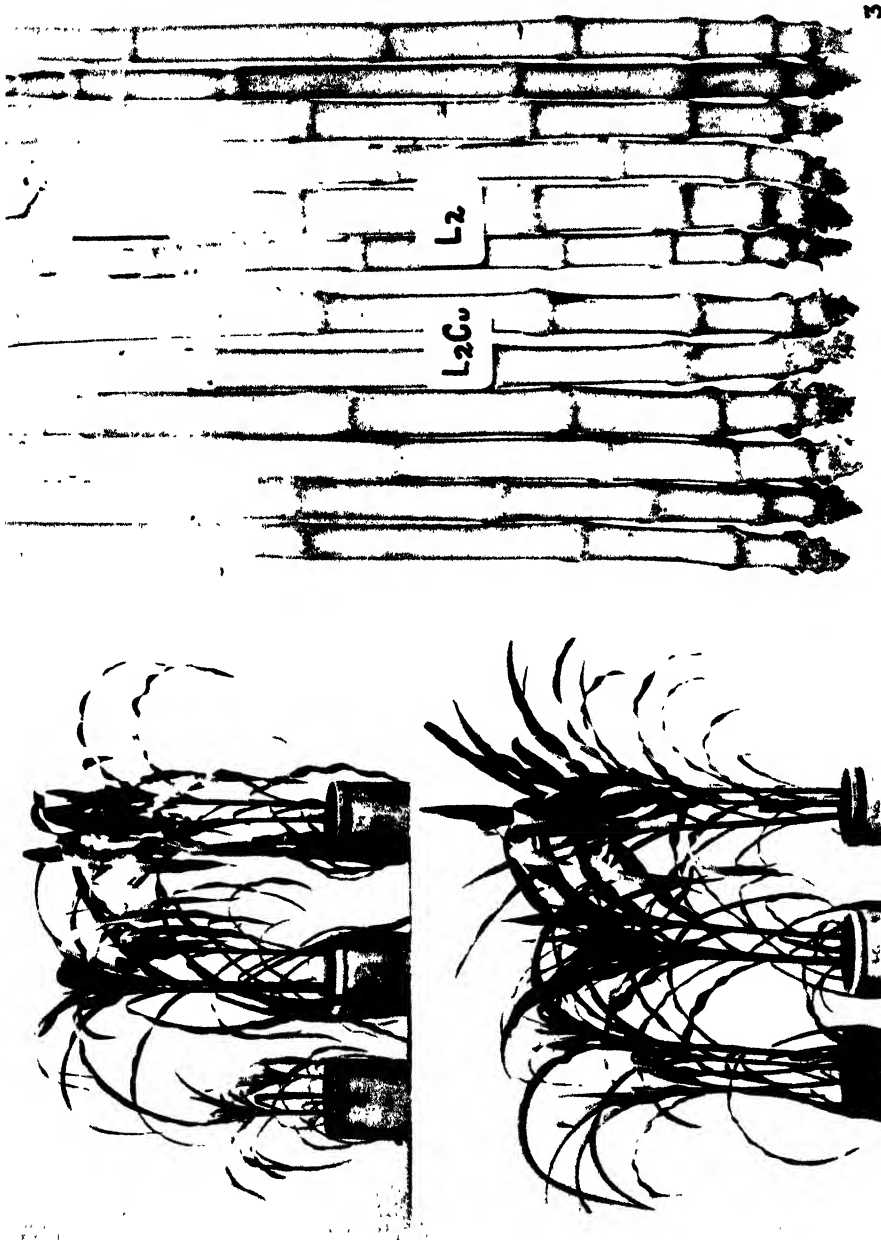
## PLATE 1

## EFFECT OF COPPER SULFATE ON IRON ABSORPTION BY CORN PLANTS

FIG. 1. Corn plants on peat soil with lime alone. Left—no lime; center—5 tons of lime per acre; right—10 tons of lime per acre.

FIG. 2. Corn plants on peat soil with lime and copper sulfate. Left—no lime; center—5 tons of lime per acre; right—10 tons of lime per acre.

FIG. 3. Iron accumulation in lower nodes of corn plants.  $L_2Cu$ —lime 10 tons and copper sulfate 1,000 pounds per acre.  $L_2$ —lime 10 tons per acre.





# THE EFFECT OF SULFUR AND PHOSPHORUS ON THE AVAILABILITY OF IRON TO PINEAPPLE AND MAIZE PLANTS<sup>1</sup>

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In 1927 the senior author conducted certain experiments on the effect of activated sulfur<sup>3</sup> on pineapple plants grown in a soil of almost neutral reaction with various commercial fertilizers added to it. The results indicated that the activated sulfur increased to some extent the acidity of the soil and also favored the development of the plants. A near-by plot treated with a mixture of this sulfur and iron oxide yielded somewhat similar results. It was thought at the time that the increased yields in both cases resulted from the liberation of water-soluble iron by the increased acidity of the soil.

Since then, these experiments have been repeated many times in soils with a different reaction and also with varying amounts of annual rainfall, the results indicating that the application of large to moderate quantities of phosphates to soils with high pH values causes depression of the growth of pineapple plants, whereas similar applications to relatively acid soils stimulate their growth and fruit yields. It was found, however, that if soils of high pH values containing moderate to large quantities of phosphates and showing depression of growth are treated either with activated sulfur or with compounds containing slowly available iron the depressed condition of the plants can be either prevented or remedied. With acid soils, however, the conditions are reversed. In this latter case moderate to high applications of phosphates have not been observed to cause depression of plant growth, nor has the addition of sulfur ever caused stimulation of growth. It has been found (13a) that the addition of sulfur to naturally acid soils causes depression of plant growth, indicating that sulfur itself is not the causative agent of stimulation but rather the acidity which it produces in neutral soils and its subsequent effects on the solubility of various minerals and especially iron.

It is the opinion of various investigators, in respect to Hawaiian soils, that

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<sup>2</sup> The authors are under obligation to Messrs. Karsten Thot and A. T. Longley of the Hawaiian Pineapple Company and to Messrs. Norman King and F. A. I. Bowers of the experiment station for their cordial cooperation throughout these studies.

<sup>3</sup> This sulfur has been prepared by the senior author by mixing flowers of sulfur with a 4 per cent solution of commercial caustic potash and then subjecting the mixture for 4 hours to a temperature of 80° to 90°C. by means of a jet of steam.



the quantity of annual rainfall which different soils have received in past geological times has influenced tremendously their acidity and other physico-chemical properties. The acidity of soils, for example, with 80 to 100 inches of annual rainfall, varies between pH 3.6 and 4.2, whereas those with a rainfall of 20 to 40 inches varies between pH 5.8 and 7.2. The effect of different quantities of average annual rainfall on the resulting acidity of the soil may be better seen in figure 1.

One can readily see in figure 1 that the quantity of average annual rainfall received by different soils is the major factor, that is, in the development of their respective acidity through the unequal leaching of the water-soluble bases.

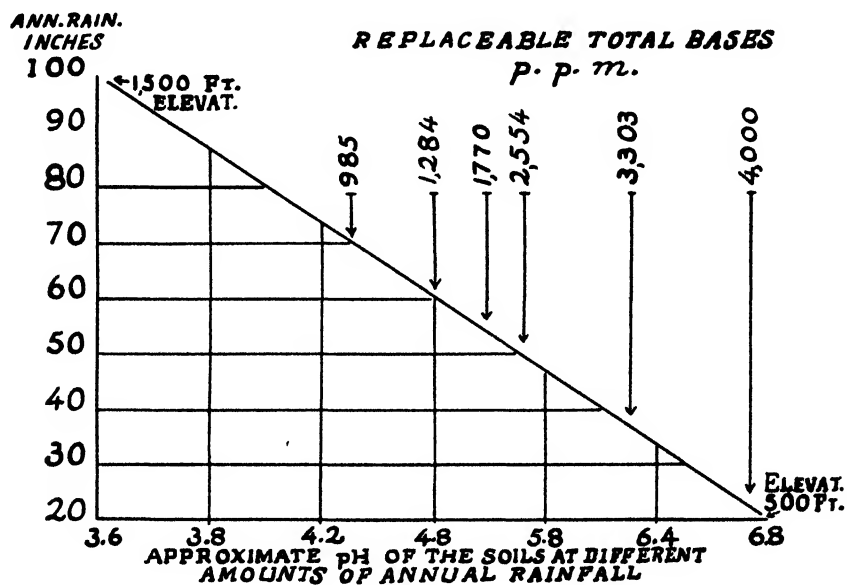


FIG. 1. AVERAGE ANNUAL RAINFALL, TOTAL REPLACEABLE BASES, AND pH OF SOILS ON THE SLOPE BETWEEN LOWER BRODIE AND UPPER HELEMANO DISTRICTS ON THE ISLAND OF OAHU

(Adopted from certain studies by Messrs. Hartung, Harker, and Crutchfield)

One additional feature of these soils, also, which possibly exists in other soils, is the fluctuation of the pH of their solution during dry and wet seasons of the year. It has been repeatedly observed that during the winter months the acidity is higher than during the summer months. The causes entering into this problem have not yet been studied. There are two possible explanations to account for this behavior: One is that the  $\text{H}_2\text{CO}_3$ , which is likely to be more abundant in wet soils on account of the highly favorable conditions for the development of microorganisms, is responsible for the increased acidity. The other is the leaching effect of rain water on the basic salts of the soil, which may be washed to greater depths or washed away, leaving the acidic clay behind.

Certain of the other physicochemical properties of these soils may be studied in table 1. This table, representing the investigations of Messrs. Hartung, Harker, and Crutchfield, is introduced here to show certain of the peculiarities of the soils under discussion.

It can be readily seen in table 1 that as the annual rainfall decreases the pH and replaceable bases increase and *vice versa*. It is quite evident that the factor which is directly responsible for decreasing the replaceable base content of soils, namely rainfall, is also indirectly responsible for the increase of hydrogen ions. McIntire (9) in his studies on certain interrelations between calcium and magnesium on the one hand and potassium and hydrogen ions on the

TABLE 1

*The influence of annual rainfall on the physicochemical properties of certain Hawaiian soils*

FIELD	ANNUAL RAIN	pH	REPLACEABLE		SULFUR TOTAL	PHOSPHORUS		REPLACEABLE						
			Iron	Aluminum		Total	Citric acid	Potash	Potassium	Calcium	Magnesium	Total bases		
	<i>inches</i>			<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>		
Helemano	19	70	4.3	Trace	433	5,790	640	9	12,670	106	184	37	327	
	21	70	4.7	Trace	467	*	930	25	17,010	105	258	55	418	
	18	70	4.4	Trace	383	624	695	12	17,122	128	264	144	536	
	26	55	4.7	Trace	5	1,300	1,530	18	13,000	372	696	193	1,261	
	41	50	5.3	Trace	90	288	1,287	22	14,200	305	943	269	1,517	
	33	45	5.8	None	37	1,770	850	10	14,900	515	1,172	280	1,967	
	27	40	6.0	None	3	2,200	920	13	13,800	400	1,528	258	2,186	
Waialua	{	35	5.9	None	0	1,300	1,270	13	14,900	560	1,656	301	2,517	
		75	35	6.0	None	24	1,770	1,480	21	13,700	601	1,885	325	2,811
		77	30	6.3	None	31	2,200	1,130	13	14,400	486	2,332	539	3,357
Lanai	20	7.0	None	38	*	1,702	16	10,000	552	2,853	506	3,911		

\* Not determined.

other hand, during soil formation, concludes as follows: "The greater loss of Ca and Mg and the accumulation of potash compounds and the subsequent accumulation of hydrogen ions during the process of soil formation show that the minimum losses of potash occur during alkaline conditions." These observations of McIntire are to a considerable extent true with the majority of pineapple soils. In respect to the effect of rainfall on the relative acidity of soils, Thomas (17), basing his conclusions on the reaction of various soil samples from different sections of the United States, claims that "arid soils have higher pH values than humid soils."

The quantitative determination of iron on soils with a different initial pH was made without air drying or oven drying the soil. The soil samples were taken

from the field with an initial moisture content of 20 to 30 per cent. The moisture content was determined on small but representative samples and the soil extracted with water in the ratio of 1:3. Only the clear supernatant liquid,

TABLE 2  
*Solubility of iron in certain soils of pineapple fields at different pH values*

pH	IRON IN SOILS		
	Helemano 21	Kemoo 49A	Brodie 88
	p.p.m.	p.p.m.	p.p.m.
3.4	0.08	0.07	0.06
4.2	0.06	0.05	0.04
4.6	0.04	0.03	0.03
5.1	0.02	<0.02	<0.02
6.2	0.01	<0.01	<0.01

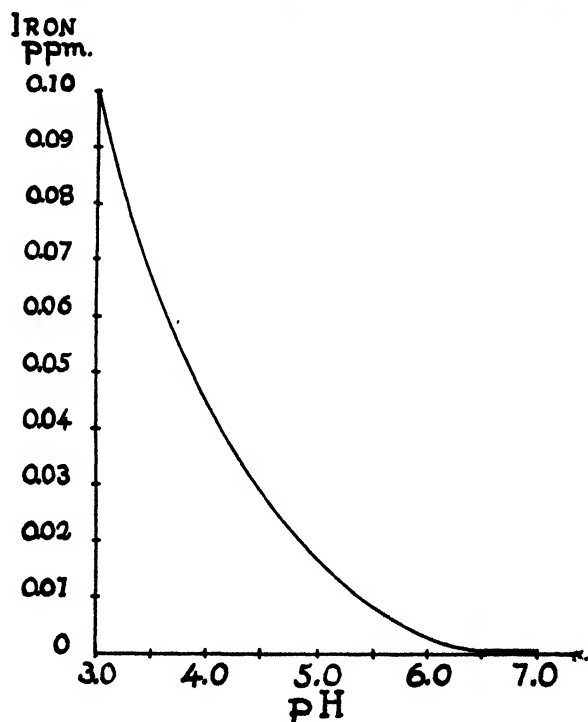


FIG. 2. WATER-SOLUBLE IRON CONCENTRATIONS AT DIFFERENT pH VALUES OF CERTAIN SOILS OF PINEAPPLE FIELDS

free from colloidal material as much as possible, was decanted and filtered through hardened filter paper. About 1 liter of filtrate which was practically free from colloidal suspension was evaporated to about 50 and then digested with HCl for colorimetric iron determination, by the method of Berman (a1).

The results, which are the average of many soils, are represented in table 2 and figure 2.

One can readily observe in figure 2 that the water-soluble iron is influenced considerably by the hydrogen-ion concentration of the soil, being relatively soluble at high acidities and either insoluble or only slightly soluble at neutrality. Turner (18) has obtained practically similar results with the tropical soils of Trinidad.

# EXPERIMENTAL STUDIES

Certain experiments dealing with the effect of different quantities of sulfur, iron, copper, potassium, phosphorus, and nitrogen on the development of pineapple plants were started in 1927 in H. P. Co. field 49A on the island of

TABLE 3  
*Fertilizer treatments (pounds per acre) and yields obtained from pineapple plants*

FIELD	PLOT	NITRO- GEN	PHOS- PHORUS	POTAS- SIUM	SULFUR	SULFUR PLUS IRON	YIELDS	DIFFER- ENCE	pH
							<i>per cent</i>	<i>per cent</i>	
H. P. Co. 49A	Check	86	49	45	..	...	100 0	.. ..	6 6
	I-D-1	....	.	...	200	...	102 3	+2.3	6 0
	I-D-2	..	..		400	...	101 2	+1.2	5 8
	I-D-3	.. .	..	..	600	...	101 2	+1 2	5 6
	I-E-1	24 7	30	75	..	...	92 7	-7 3	6.6
	I-E-2	24 7	30	150	.	...	89 0	-11 0	6 5
	I-E-3	24 7	30	300	...	...	92 0	-8 0	6.4
	I-F-1	49 0	60	75	...	...	96.8	-3 2	6 5
	I-F-2	49.0	60	150	...	...	97.2	-2 8	6 4
	I-F-3	49 0	60	300	...	...	97.7	-2.3	6 3
	III-A-1	45.0	30	100	...	800	100 5	+0 5	5 7
	III-A-2	90 0	60	100	...	800	105.0	+5.0	5.6
	III-A-3	160 0	120	100	.	800	102.3	+2 3	5.5

Oahu with an average annual rainfall of about 35 inches. The field, before application of the fertilizers, indicated in table 3, had been treated uniformly with 1,500 pounds of a complete fertilizer of the formula  $N11\frac{1}{2}-P6\frac{1}{2}-K5$  per cent. The yields which were obtained in the plant crop in the succeeding 18 months are reported in table 3. The so-called first and second ratoon yields, namely the second and third crops, are not reported here because the plants of practically all the plots, except those of I-D-1, -2, and -3 and III-A-1, -2, and -3, died with wilt. Only plots free from wilt were selected for obtaining the yields reported in table 3.

Table 3 shows that the addition of activated sulfur to a soil with an initial reaction of pH 6.6 was very beneficial to the plants grown therein. It shows

also, that increasing phosphorus did not benefit the plants. This is also true in regard to potassium, as this soil, being one with an average annual rainfall of about 35 inches, contains an ample supply of this element.

This experiment was repeated the following year in H. P. Co. field 78 with practically identical results, showing that heavy applications of phosphorus, unless accompanied with similar applications of activated sulfur, exerted a depressing effect on plant growth and yields.

The sulfur treated plants in both experiments were greener than the untreated ones, their vigor was greater, and in the following year's crop they produced greater yields.

An experiment along similar lines was conducted in 1930 to 1931 in two different fields with almost similar soil types and quantity of annual rainfall.

TABLE 4  
*Fertilizer applications and growth response (fruit weight) of pineapple plants*

TREATMENTS PER ACRE					H. P. CO. FIELD 83 BRODIE		H. P. CO. FIELD 82 (4511 KEMOO)		DIFFER- ENCES IN THE PER CENT YIELD
Plot	Sulfur	Nitrogen	Phos- phorus	Potassium	Fruit weight	Per cent yield	Fruit weight	Per cent yield	
	lbs.	lbs.	lbs.	lbs.	lbs.		lbs.		
B	.....	376	179 7	201.0	4.23	100 0	4 2	100 0	0
G	.....	506	179.7	201.0	4 45	105 2	4 4	105.0	0 2
C	634	376	179 7	201 0	4.30	101 6	4.3	102 4	0 8
D	634	506	179.7	201.0	4 45	105.2	4 4	105 0	0 2
E	634	634	179 7	201 0	4 75	112.3	4 7	112 0	0 3
K	634	428	249.0	201.0	4.32	102.1	4.3	102.4	0.3
L	634	558	249 0	201 0	4.55	107.5	4 5	107.1	0 4
H	1,267	376	179.7	201.0	4 35	102.8	4.3	102 4	0.4
I	1,267	506	179.7	201.0	4.48	107 5	4.5	107.1	0 4
J	1,267	636	179.7	201 0	4 63	109 5	4 6	109.5	0
M	1,267	428	249.0	201.0	4 37	103 3	4.4	104 8	1.5
N	1,267	558	249 0	201 0	4 43	104 7	4 4	104 8	0 1

These were H. P. Co. fields 83 (Brodie) and 82 (Kemoo). The results obtained are recorded in table 4.

It will be observed that the results in table 4, representing the yields obtained from two different fields with identical treatments and during the same growth periods, are not only in perfect harmony between themselves but they also agree on general principles with those in table 3. The outstanding features of these experiments are: (a) the interfering effect of phosphates on the solubility of iron and its availability to plants at high pH values, namely between 6.0 and 7.0, which is reflected on the plants by depression of their growth, and (b) the stimulating effect of sulfur on the growth of plants and improvement of the color of their leaf pigments by increasing the initial acidity of the soil whereby iron becomes soluble and available to plants.

The phenomenon of iron unavailability to plants as a result of high phosphate

applications to soils has been further studied in the greenhouse on corn plants. These plants were selected because of their great rate of growth and susceptibility to iron deficiency. The results obtained are illuminating in that they show the effect of relatively high and low concentrations of phosphates on the response of plants grown in solution cultures containing high as well as low initial concentrations of iron and at pH values ranging between 6.0 and 7.0. The results of these studies are presented in table 5.

TABLE 5

*Growth of maize plants at different initial iron and phosphate concentrations and pH between 6 and 7*

INITIAL PHOSPHATE CONCENTRATIONS 31.0 P.P.M.				INITIAL PHOSPHATE CONCENTRATIONS 3.1 P.P.M.			
Culture number	Initial iron concentrations	Plant weight	pH	Culture number	Initial iron concentrations	Plant weight	pH
	p.p.m.	gm			p.p.m.	gm	
I-A-2	7 0	22 5	Not adjusted 6 8-6 4	I-B-2	7 0	33 0	Not adjusted 6 8-6 4
I-A-3	14 0	28 3		I-B-3	14 0	36 3	
I-A-4	28 0	45 3		I-B-4	28 0	47 7	
I-A-5	35 0	55 6		I-B-5	35 0	49 0	
I-C-2	7 0	25 7	Adjusted 6 6-6 3	I-D-2	7 0	30 0	Adjusted 6 8-6 4
I-C-3	14 0	37 9		I-D-3	14 0	35 4	
I-C-4	28 0	46 6		I-D-4	28 0	45 5	
I-C-5	35 0	53 0		I-D-5	35 0	41 0	
I-E-2	7 0	Dead*	Not adjusted 6 7-7 1	I-F-2	7 0	30 8	Not adjusted 6 8-6 4
I-E-3	14 0	Dead*		I-F-3	14 0	39 4	
I-E-4	28 0	30 0		I-F-4	28 0	45 4	
I-E-5	35 0	42 0		I-F-5	35 0	47 5	
I-G-2	7 0	Dead*	Adjusted 7 0-6 6	I-H-2	7 0	30 0	Adjusted 6 8-6 4
I-G-3	14 0	Dead*		I-H-3	14 0	36 6	
I-G-4	28 0	40 0		I-H-4	28 0	43 6	
I-G-5	35 0	40 0		I-H-5	35 0	43 3	

\* The plants of these series died as a result of the development of chlorosis in the early stages of their growth. The chlorotic condition of these and all the plants in general became highly intensified on account of the high temperature of the greenhouse.

The results in table 5 show that the plants at low phosphate and iron concentrations developed better than those at high phosphate and low iron concentrations. The plants at high iron concentrations developed well regardless of the initial phosphate concentration, the results being slightly in favor of high iron and high phosphate concentrations. The results show very clearly that high phosphate concentrations depress plant growth at pH values between 6.0 and 7.0; (a) because of the low solubility of iron and (b) because of the interfering effect of phosphates on iron solubility at high pH values.

Certain other studies in respect to the behavior of pineapple and maize plan in solution cultures and in naturally acid soils containing moderate to high concentrations of phosphates have yielded results indicating that the effect of phosphates in such soils is highly beneficial to the development of plants. The explanation of the beneficial effect of phosphates is the same as that mentioned, namely, that in acid solutions phosphates do not interfere with the solubility of iron because hydrogen ions replace sufficient iron for the requirements of plants. It is possible that iron in acid solutions exists either as ferric or ferrous phosphate, in which case the plant can absorb both ions. Teakle's (16) studies on the effect of reaction and cation concentrations on the solubility of phosphates indicate that "iron in combination with phosphate is least soluble under very acid conditions corresponding to pH 3.0. Under conditions of less acidity the hydroxide is formed at the expense of iron phosphate with the liberation of phosphate ions, as the solubility product of  $\text{Fe}^{+++}$  and  $\text{OH}^-$  is of such a magnitude as to favor the reaction in this direction." The problem of iron deficiency resulting through the application of relatively large quantities of phosphates can be studied much more easily and with a greater degree of confidence by biological rather than by chemical methods, because the quantities of iron necessary to maintain the normal requirements of plants range in concentrations probably between 0.01 and 0.001 p.p.m.

#### DISCUSSION OF RESULTS

The results presented in tables 1, 3, 4, and 5 make quite evident the fact that moderate to high applications of phosphates to pineapple soils with an initial reaction ranging between pH 6.0 and 7.0 may cause depression of growth as well as decrease in the yields, whereas the same quantities of phosphates applied to soils with an initial reaction ranging between 3.5 and 5.8 (referring in this case to certain experiments now under way) may cause stimulation of plant growth and increase of yields. It has been shown that the addition of moderate quantities of activated sulfur to soils with moderate to high concentrations of phosphate fertilizers and with a reaction ranging between pH 6 and 7 increases the growth and yields of plants. In cases, however, where relatively large quantities of sulfur have been applied and where, either because of high acidity or of certain by-products of sulfur, the plants show depression of growth, this condition can be remedied by the addition of phosphates.

Table 4 shows that fruit yields have increased with increments of nitrogen. In respect to other treatments, namely phosphorus and sulfur, the results may be interpreted as follows:

Fruit yields increased with increments of nitrogen.

Sulfur has increased the yields at low nitrogen concentrations. At high nitrogen concentrations, however, sulfur did not cause yield increases on account of the acidifying effect of the sulfur of the nitrogenous fertilizer  $[(\text{NH}_4)_2\text{SO}_4]$  which probably released iron in sufficient concentration for the requirements of the plants. The beneficial effect of sulfur is therefore limited to its action in acidifying the soil and releasing iron. If such a process can be accom-

plished by some other reagents, as in this case with ammonium sulfate, sulfur itself loses its beneficial properties.

The addition of relatively larger quantities of phosphates to soils with moderate quantities of sulfur decreases yields.

The addition of relatively large quantities of sulfur to soils containing moderate quantities of phosphates increases yields.

The addition of relatively large quantities of sulfur to soils containing also large quantities of phosphates decreases yields.

The soils under consideration in table 4 have a pH ranging between 6.4 and 7.0 and an annual rainfall of 20 to 35 inches. They contain a fair concentration of insoluble and replaceable potassium, calcium, and magnesium and are also well supplied with sulfur and various other elements essential for normal growth. The response of such soils to sulfur treatments can not be claimed on the basis of sulfur deficiency but on that of increased acidity which is instrumental in making iron available.

Pitz (11) in his studies on the effect of elemental sulfur in soils claims that "large amounts to an extent of 0.05 per cent produce an increase in ammonification which is accompanied by a parallel decrease in nitrate formation." Eaton (2) observed that "flowers of sulfur, sodium sulfate and gypsum caused increased dry weights of sweet corn of 35-66 per cent. Larger amounts, however, gave no increases." Reimer and Tartar (12) in their studies of sulfur on certain Oregon soils found that "flowers of sulfur, iron sulfate and superphosphate each produced an increase of fully 100 per cent in yield of alfalfa and greatly improved the color of the plants, whereas rock phosphate, monocalcic phosphate, nitrate of soda muriate of potash and lime in the same field had no influence on the crops. The soils on which these experiments have been conducted are well supplied with potassium, calcium, magnesium and iron, and contain but limited amounts of sulfur. None of them are acid and none contains noticeable amounts of alkali." Stewart (15) in his studies on the relation of sulfur to soil fertility claims that "The only conclusion that can be drawn from the available data on this subject is that, under humid conditions, sulfur need not be added to the soil as a plant food." If in connection with the statement of Stewart we consider also that of Thomas that "arid soils have high pH values and humid soils low values" we come back to our original assertion that "the addition of sulfur to acid soils inhibits plant growth, whereas, to neutral soils it stimulates plant growth." The literature on sulfur bears out these facts: (a) that sulfur may stimulate plant growth in soils where sulfur deficiency exists and (b) that it may stimulate plant growth by increasing the acidity of neutral or alkaline soils where iron and possibly certain other elements are unavailable because of extremely low solubility or total insolubility. It is possible that both these conditions have operated in the experiments of Reimer and Tartar as long as they were dealing with practically neutral soils. In regard to Hawaiian soils, sulfur deficiency can not be claimed according to the results in table 1. The stimulating effect observed can be totally claimed



for the increased acidity which rendered iron more soluble and available to the plants.

In respect to phosphates, the available literature indicates that the time of application, according to Gericke (3), and the reaction of the soil play a very important rôle in the favorable response of plants to phosphate treatments. The report of Ross, Jacob, and Beeson (13), which is a compilation of investigations on the efficiency of various phosphatic fertilizers in different sections of the United States, indicates that "the efficiency of heavily ammoniated superphosphate, tricalcium phosphate, steamed bone meal and basic slag is greater on acid soils than on soils that approach neutrality. . . . About 90 per cent of the soils in the sections of the country consuming 75 per cent of the phosphatic fertilizers sold in the United States have pH values below 6.0." Waltman (19) in his studies on the effect of H-ion concentration on the growth of strawberries in sand and in soil states that ". . . strawberries need a relatively large amount of phosphorus and that the reaction of the soil may influence materially the availability of this substance. It appears that an acid reaction is favorable to the utilization of phosphorus by the plants." Krauss (6) in his studies on the effect of phosphates on corn plants found that the humid and acid soils of Haiku, Maui, Territory of Hawaii, responded very favorably to phosphate treatments. Davidson (1) in his studies on phosphorus absorption by wheat seedlings claims that ". . . more phosphorus was absorbed by the seedlings from the solutions with initial pH values of 5.0 and lower than from those with pH values of 6.0 and 7.0. As all potassium salts of phosphoric acid are soluble [these being the kind of salts he used in these studies], this tends to show that the physiological availability of phosphorus depends upon the hydrogen-ion concentration of the medium." The recent work of McGeorge (7, 8) shows that an increased acidity, namely from pH 6.8 to 6.0, by means of carbonic acid increased somewhat the solubility of phosphates and favored also the development of plants. It is to be regretted that McGeorge has not studied also in this connection the effect of the increased acidity on the solubility and availability of iron to his plants. Heck and Truog (5) have reported recently on their studies of phosphate fixation in soils that ". . . If phosphates are applied to acid soils lacking in lime they will be fixed largely as basic iron and aluminum phosphates. Soils of high fixing power, that is, those containing an abundance of readily available lime or certain hydrated oxides of iron and aluminum, fix the soluble phosphates very quickly. . . ." Willis (20) comments as follows in his studies on the effect of liming soils on the availability of manganese and iron:

Liming at rates to produce pH values of the soil less than 7.9 is not likely to produce deficiency of iron for plants except where the concurrent absorption of lime by the plant causes physiological disturbances that make the iron less efficiently utilized. In the presence of strong oxidants, such as manganese dioxide, the reaction at which symptoms of a deficiency are produced may be as acid as pH 6.0, although there is no certainty that the effect in such cases is not accentuated by the absorption of abnormal quantities of manganese. . . . There is no

satisfactory evidence that a deficiency of iron is solely responsible for the symptoms shown by susceptible crops, such as pineapples, rice and some trees, except as a possible reduction in the concentration of soluble iron in the soil consequent to liming is associated with an unfavorable effect of the lime or other soil component on the assimilation or translocation or utilization of the iron by or in the plant.

The statements of Willis are quite logical and fit the observed facts very well as far as Hawaiian soils and pineapples are concerned. As far as his last statement is concerned, however, the authors have certain unpublished evidence indicating that the  $\text{Ca}^{++}$ , *per se*, as  $\text{Ca}(\text{NO}_3)_2$  in acid solutions and in the presence of ample quantities of iron for the normal growth of pineapple plants does not interfere very appreciably with iron absorption. Only in cases of low acidity or neutrality, and only as  $\text{CaCO}_3$  or  $\text{Ca H PO}_4$ , has  $\text{Ca}^{++}$  been found to interfere with the solubility and availability of iron. One is not justified in attributing the insolubility of iron under such conditions to the  $\text{Ca}^{++}$ , but rather to such other ions as  $\text{OH}^-$  or  $\text{PO}_4^{--}$ .

Certain conditions which have been observed to influence greatly the effectiveness of iron in the tissues of pineapple plants are (a) the form of nitrogenous salts absorbed by the plants, and (b) the salt content, especially base content, of the solution or soil culture. In respect to the former condition, it has been observed that ammonium sulfate favors a better growth of pineapple plants than either potassium or sodium nitrate. Analyses of the tissues of pineapple plants grown with ammonium sulfate by Stewart, Thomas, and Horner (14) and of corn plants by the authors (13b) have indicated that the presence of ammonium in the solution culture inhibits the partial absorption of potassium, calcium, and magnesium, showing that ammonium cultured plants contained about one-half as much potassium, calcium, and magnesium as nitrate cultured plants. Pearsall and Ewing (10) in their studies on the relation of nitrogen metabolism to plant succulence, conclude as follows: "... in plants supplied with abundant nitrates, amino-acids tend to accumulate, and these influence the metabolism of the plants in such a way that acid production is reduced, with a consequent reduction in the hydrogen-ion concentration of the sap." Regardless of the exact biochemical processes involved during the assimilation of nitrates the fact remains that they exert some influence on the properties of the cell sap which possibly affects the stability of water-soluble iron and its mobility in the tissues. Further studies on the effect of iron in plant growth and nutrition will be presented in a paper to follow.

#### CONCLUSIONS

The conclusions to be drawn from the foregoing studies are as follows:

Pineapple soils with a high annual rainfall are relatively very acid, because they are lacking in such water-soluble bases as potassium, calcium, and magnesium. This condition is brought about by the fact that most of the water-soluble minerals of these bases have been leached by rain and replaced with hydrogen ions. Pineapple plants grown in such soils respond very favorably to phosphate fertilizers. This response is due to the slight solubility of phosphates

under such conditions, as they form various relatively insoluble compounds with either iron or aluminum which release water-soluble iron and phosphorus in quantities very small but sufficient for the normal development of plants. Thus, both iron and phosphorus can be obtained by plants in small but sufficient quantities.

Pineapple soils with a low annual rainfall have relatively high pH values, namely 6.0 to 7.0. Their high pH values are due to their high content of such bases as potassium, calcium, and magnesium in combination with either strong or very weak acids. They do not respond to high or moderate applications of phosphates on account of their high pH values, at which phosphates in combination with either K, Ca, or Mg are relatively soluble and interfering with the solubility of the small traces of water-soluble iron which exist under such conditions. If such soils are rendered more acid with sulfur or with acid fertilizers, or are supplied with forms of slowly available iron they stimulate plant growth very appreciably even in the presence of moderate applications of phosphates.

The solubility of iron and its availability to plants should be watched very carefully in the application of phosphate fertilizers. The writers have found that the biological method of determining iron deficiency, at least with certain plants, is of greater sensitivity than the various chemical methods recommended.<sup>4</sup>

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# THE RELATION BETWEEN REPLACEABLE POTASSIUM AND FIELD RESPONSE TO POTASH IN HAWAIIAN SOILS<sup>1</sup>

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A number of excellent fertilizer experiments on pineapples have been conducted in the Territory of Hawaii. The yield increases caused by potash application have in general been negligible, with a few exceptions. This paper contains data on the more exact field experiments and correlates field response with chemical analyses of the soils on which the experiments were installed.

## REVIEW OF LITERATURE

Base exchangeable or replaceable potash serves as a reservoir to replenish the soil solution with potassium if the concentration in it is lowered. Several investigators, including Hissink (5), Kellner (7), Breazeale (1), and Martin (9), have shown that plants can absorb and utilize potassium coming from the replaceable complex.

Plants are not entirely dependent on the water-soluble and replaceable potash. During the growing period, potash from other forms becomes available. Thus, Gedroiz (4), working with a soil from which all but traces of replaceable potash had been removed, was able to grow a satisfactory crop of oats, mustard, and buckwheat. Martin states (9, p. 131), "The bulk of the evidence at hand seems to indicate that the barley crops have either obtained part of their potassium from the non-replaceable form; or if it has all been absorbed from the replaceable complex, directly or indirectly, there has been a partial replenishment of the potassium in this complex from the non-replaceable form."

Frazer (3) presented data indicating that the amount of replaceable potash in his soils initially, minus that at the end of the experiment, was less than that contained in a crop of corn and of sorghum grown on these soils.

More recently, Hoagland and Martin (6) stated that, "Of the total potassium absorbed by plants over a long period of intensive cropping, the greater part may be derived from the non-replaceable potassium fraction of the soil. This fraction may or may not be capable of supplying potassium to the plants at adequate rates, depending on the nature of the soil." These authors grew barley and tomatoes on several soils with and without potassium fertilization.

One of the first investigators to demonstrate the relationship between the replaceable potash content of a soil and its crop productivity was Nostitz (11). In his experiments of 1922, soils deprived of their replaceable potash content by leaching with ammonium solutions failed to grow as good crops of barley and mustard as did the untreated soil and the soil saturated with calcium, magnesium, and potassium. In 1929, Frazer obtained a correlation

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<sup>2</sup> Chemist. The author is indebted to Kauai Pineapple Co., Ltd.; Libby, McNeill and Libby; Hawaiian Pineapple Co., Ltd.; Maui Pineapple Co., Ltd.; and Pacific Guano and Fertilizer Co. for cooperation and permission to publish field results.

coefficient of  $0.91 \pm 0.012$  between the replaceable potash content of his 41 soils and that removed by two crops grown on these soils.

Sewell and Perkins (13) on the basis of long continued corn yields in a number of counties in Kansas, concluded that a high content of exchangeable potassium seemed to be directly related to good corn yields.

#### EXPERIMENTAL

A number of field experiments with pineapples in which the quantity of potassium applied has been varied, have been conducted by various pineapple companies and by the Pacific Guano and Fertilizer Company, or with the experiment station coöperating. In general, the fertilizer experiments reported have consisted of three to five levels of application of nitrogen, phosphorus, and potassium. Each treatment was replicated three to six times.

TABLE 1

*A list of field experiments with varying potash fertilization on pineapples*

EXPERIMENT		LABORATORY NUMBER OF SOIL	M. E. REPLACE- ABLE K <sub>2</sub> O PER 100 GM.	PER CENT YIELD INCREASE	STANDARD ERROR OF A TREATMENT IN PER CENT OF MEAN	HARVEST YEAR
Letter (Fig 1)	Company number					
G	KP-4	876	0.11	44.4	....	1929
H	HP-18	671-702	0.17	11.6	2.95	1926
A	KP-3	875	0.20	13.2	1.15	1929
B	KP-5	988	0.47	4.4	4.32	1927
E	L-98-C	1597	0.47	None	3.64	1930
M	KP-9	616	0.64	None	1.43	1932
J	HP-88	R-21	0.90	None	....	1926
F	L-98-D	1595	0.92	0.5	...	1930
D	L-98-H	1594	0.94	None	....	1930
C	L-98-G	918	1.01	None	....	1930
N	Haleakala	{ 1596 6152-4 }	1.05	None	...	1930
I	HP-85	917	1.08	2.3	2.72	1926
K	HP-13	R-13-5	1.19	2.9	2.84	1928
L	HP-14	R-13-6	1.24	None	2.12	1928

The treatments in which potash was varied received high and uniform applications of nitrogen and phosphorus. Experiment M, table 1, was a triangle system experiment such as described by Schreiner and Skinner (12) and in that respect differs from the remainder of the experiments. There was no response to potassium or phosphorus in this experiment. A list of the field experiments together with pertinent data appears in table 1.

#### *Accuracy of field experiments*

In the case of a number of experiments, individual plot records are available. By the use of the method of calculation outlined by Fisher and Wishart (2), the standard errors of a number of these experiments have been calculated.

The plot size has varied from 0.1 to 0.5 acre containing 1,000 to 8,000 fruits per plot. Variance is consequently very small within plots, and relatively large between plots. The results are given in table 1.

Harvest records for each individual experiment are shown in figure 1. The dotted lines join yield points. The yield equation was calculated by the method of Magistad, Farden, and Lambert (8). The curve expressed by the yield equation is shown as a continuous line (as in experiment G).

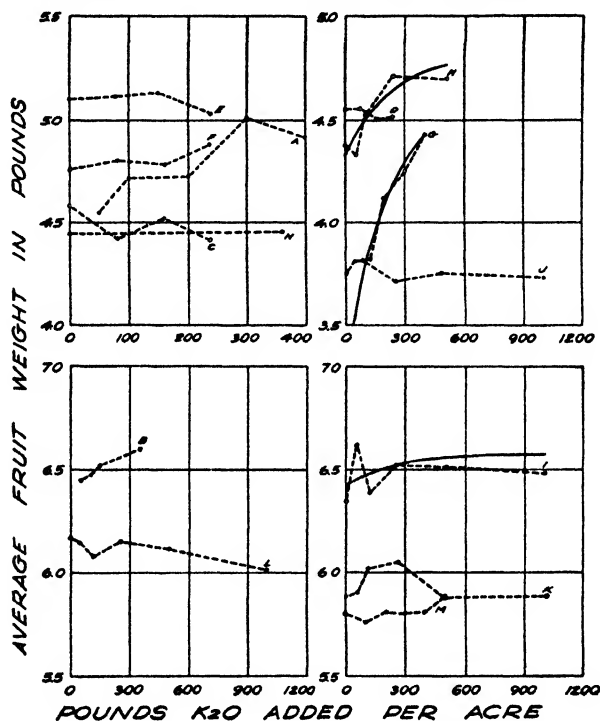


FIG. 1. HARVEST YIELDS OF PINEAPPLE FRUITS IN FIELD EXPERIMENTS WITH VARYING POTASH FERTILIZATION

A key to the experiments is given in table 1

On the basis of the yield equation, the values in the column in table 1 headed "per cent yield increase" have been calculated as follows: The yield increase possible by heavy potash fertilization,  $A$ , divided by the yield without potash application ( $M - A$ ), on a percentage basis constitutes the per cent yield increase.

When viewed in the light of standard errors, only three experiments have shown a reliable yield increase; namely,  $A$ ,  $G$ , and  $H$ .

#### *Determination of replaceable potash*

Composite soil samples were taken from each experiment site for the determination of replaceable potassium. Experiments installed since 1929 have



been sampled prior to installation. In the case of the older experiments it was necessary to obtain soil samples between the beds of the check plots, where it is believed the quantity of replaceable potassium has not changed greatly since installation of the experiment.

Replaceable potassium was determined by leaching with normal ammonium chloride. After separation from conflicting cations it was precipitated as the chlorplatinate. In one investigation to determine the sampling error, seven composite soil samples were analyzed and yielded a standard deviation of a single determination of 15 per cent of the mean. Most of the values recorded in table 1 have a smaller standard deviation than this because the composites were made with greater care and because in some cases a number of samples were taken and analyzed.

### *Results*

The data in table 1 show a decided relationship between content of replaceable potassium and response to added potash fertilizers and forcibly indicate lack of response to added potash when the quantity present in the soil exceeds 0.4 to 0.5 m. e. per 100 gm. of dry soil. Under Hawaiian conditions, with 2,400,000 pounds of soil per surface acre foot, this is equal to about 500 pounds of  $K_2O$  per acre foot. This value for a tropical crop attaining maturity in 18 to 24 months is in good agreement with the results of Mehlich, Truog, and Fred, who obtained no yield increase in the case of alfalfa when the soil contained 15 mgm. of  $K_2O$  (0.386 m. e.) per 100 gm. of soil.

As a result of the generalization that further yield increases cannot be obtained by potash fertilization on soils having over 0.5 m. e. of replaceable potash per 100 gm. of soil, potash has been omitted from the fertilizer applied to about 70 per cent of the Hawaiian pineapple soils.

### SUMMARY

Results of 14 field experiments conducted with pineapples in which potash was added in quantities up to 1,000 pounds of  $K_2O$  per acre, are given in the form of a graph, and the yield increases are tabulated. Soils which contained over 0.5 m. e. of replaceable potassium per 100 gm. of soil gave no yield increases with added potash fertilization.

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# A NEW METHOD OF DETERMINING BASE EXCHANGE CAPACITY OF SOILS

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The term "base exchange capacity" is rather loosely applied in soil literature. Very often it is confused with saturation capacity and not infrequently identified with total exchangeable bases. "Base exchange capacity" refers to the power of the soil to combine with bases in such a manner that they cannot be easily removed by simple leaching with water; but they can be readily exchanged by an equivalent amount of other bases. In other words, it represents a limit beyond which if bases are added to the soil they would be easily hydrolyzed.

Base exchange capacity of a soil can be determined by adding excess of base to be followed by exhaustive leaching with a neutral salt until all the exchangeable ions have been replaced by the basic portion of the neutral salt. The excess of the salt is then washed out with water or alcohol, the soil leached with another neutral salt, and the displaced ions are determined in the filtrate. The success of the method and the choice of the neutral salt depend upon the ease with which a particular ion can be determined analytically.

So far the only salt extensively used for this purpose is  $\text{NH}_4\text{Cl}$  (1). This is because of the comparative simplicity of the determination of combined ammonia which can be made in the presence of the soil. However, the process of washing the excess of  $\text{NH}_4\text{Cl}$  before the final estimation is not only tedious but liable to error because of the difficulty of carrying it to completion.

Methods of analysis that can give quantitative values for the fundamental characteristics of the exchange complex in soils are highly desirable, for these must form the basis of any comprehensive system of genetic classification of soils. Following the perfection of a simple method for the estimation of exchangeable Na in soils (5), a method for the estimation of base exchange capacity of soils was devised. It consists in saturating the soil with Na by treatment with NaCl and displacing the combined Na as hydroxide by leaching with  $\text{Ba}(\text{OH})_2$ . The following is a detailed description of the proposed method.

Portions of 10 to 20 gm. of soil are treated with 100 cc. of approximately 0.05 *N* NaOH in *N* NaCl solution, filtered, and leached with 1 liter of *N* NaCl solution in 100-cc. lots. The leaching is best carried out in Buchner funnels (without suction), having filter papers fixed on by running molten wax round the edges. The leaching solution is added by means of a pipette, the soil being

stirred thereby and replacement facilitated. The soil is then stirred with about 100 cc. of a saturated solution of  $\text{Ba}(\text{OH})_2$  kept in a wash bottle. It is then leached with about 900 cc. of 0.1 *N*  $\text{Ba}(\text{OH})_2$  in 100-cc. lots. The filtrate is made up to a liter, a few drops of phenolphthalein indicator added, and  $\text{CO}_2$  passed until the solution just becomes colorless. The precipitate of  $\text{BaCO}_3$  is allowed to settle, which it does in about half an hour. Five hundred cubic centimeters of the supernatant liquid is boiled down to about 50 cc.; carbon dioxide is again passed until the phenolphthalein color is just discharged. For this purpose it is only necessary to allow the gas to play on the surface while giving a whirling motion to the beaker containing the solution. About 25 cc. of approximately 0.5 *N* ammonia solution is then added, the liquid brought to the boiling point and filtered. The precipitate on the filter paper is washed three to four times with approximately 0.05 *N* ammonia solution. The filtrate is boiled briskly until the volume is reduced to about one half, by which time all the ammonia is driven off. Excess of standard acid is then added, boiled for a while, and back-titrated with standard alkali preferably using brom thymol blue as indicator. The results are expressed in milliequivalents per 100 gm. of soil. A great feature of the proposed method is that no leaching with water is necessary after the  $\text{NaCl}$  treatment;  $\text{Ba}(\text{OH})_2$  treatment can be made at once.

#### COMPARISON OF THE $\text{NaCl}$ METHOD WITH THE AUTHOR'S AMMONIA METHOD

In earlier publications (2, 3) the author has shown that the so-called absorption of ammonia by soils is a chemical reaction representing the neutralization of acidoid by a base. Subsequently this interaction between ammonia and soils was made the basis of a method for characterizing soil colloids and defining their exchange capacity (4). The method consisted in exposing a known weight of the soil, after exhaustive treatment with 0.05 *N*  $\text{HCl}$ , to ammonia gas in a vacuum desiccator containing normal solution of ammonia. The excess of ammonia was removed by keeping the soil over 90 per cent  $\text{H}_2\text{SO}_4$  in a vacuum desiccator for 2 days. The ammonia retained by the soil was then determined by distillation with lime in the usual way.

It will be seen that the ammonia method is radically different from the  $\text{NaCl}$  method, and it was of great interest to see how far the two agree with each other. Forty-six soils from various parts of India were examined by the two methods. The results given in table 1 show a good agreement between the two methods. The largest discrepancy occurs in laterite soils, which seem to show a lower value with the  $\text{NaCl}$  method, and in dark colored clay soils, which show a higher value.

It is apparent from the results of this investigation that the exchange complex is not appreciably altered by exhaustive treatment with 0.05 *N*  $\text{HCl}$  except in the case of heavy clay soils with a high base exchange capacity. The  $\text{NaCl}$  method is to be preferred in any case as it can be carried out in a much shorter time besides being free from the objection which could be levelled against any method involving acid treatment.

TABLE 1  
Base exchange capacity of soils by two methods

SOIL LABORATORY NUMBER P.C.	LOCALITY	CLAY CONTENT— 0.002 MM.  <i>per cent</i>	BASE EXCHANGE CAPACITY M.E. PER 100 GM. OF SOIL	
			NaCl	NH <sub>4</sub>
54	Punjab	3.2	2.46	2.8
25	United Provinces	4.0	1.92	2.1
37	Bengal	4.1*	3.98	7.6
12	Assam	7.2	4.34	4.6
20	Burma	8.1	3.44	4.4
44	Bihar	8.4	6.58	6.5
39	Bombay	8.4	12.78	11.9
16	Punjab	8.7	5.26	4.9
24	United Provinces	9.7	9.00	8.8
55	Sindh	11.0	7.86	7.8
45	Bihar	11.1	5.08	5.8
23	Burma	11.3	11.1	11.1
34	Punjab	11.3	6.72	7.0
1	Bihar	11.3	5.10	4.8
52	Bihar	11.3	7.14	6.2
36	Punjab	11.7	8.22	6.85
5	Punjab	12.3	7.77	7.32
40	Bombay	13.1	10.50	11.0
21	Burma	13.5	11.57	12.9
17	United Provinces	14.1	8.79	8.2
22	Burma	15.1	13.26	12.7
4	Punjab	15.2	8.79	8.2
50	N. W. F. Provinces	17.7	8.98	9.6
35	Punjab	18.3	9.06	11.6
14	Madras	21.5*	13.47	17.2
43	Bihar	21.6	15.72	15.3
7	Punjab	21.8	8.08	7.1
51	N. W. F. Provinces	22.2	7.08	7.6
15	Assam	22.4*	10.98	14.3
26	Mysore	22.5	4.98	5.7
18	Burma	22.6	11.80	11.7
31	Travancore	22.8	16.74	17.0
49	Bengal	23.3	16.74	17.0
8	Madras	25.2	20.03	18.7
9	Malabar	26.1	7.67	8.1
6	Bengal	28.4*	10.16	11.0
11	Madras	32.8†	27.97	23.7
10	Madras	35.6†	28.14	25.1
19	Burma	42.3†	26.48	24.05
27	Mysore	53.2†	55.66	51.3
42	Bombay	53.4†	58.06	56.3
41	Bombay	53.4†	51.54	51.7
30	Central Provinces	54.1†	57.22	57.5
13	Central Provinces	58.9†	56.64	48.0
3	Bombay	62.2†	67.71	63.06
29	Central Provinces	63.0†	53.28	49.1

\* Laterite soils.

† Dark colored clay soils.

The use of base exchange capacity per gram of clay for characterizing soil colloids has already been discussed elsewhere (4). It might be pointed out that base exchange capacity refers to a very important property of soils, and is much more significant than clay content, for whereas the latter refers to an arbitrarily fixed size of the particles, the former gives a measure of their reactivity. It is hoped that the base exchange capacity as determined by the method outlined in this paper will prove useful as a fundamental characteristic constant for classifying soils.

#### SUMMARY

A method for finding the base exchange capacity of soils has been outlined. It consists in leaching the soil with NaCl followed by displacement of the exchangeable Na as NaOH by treatment with 0.1 *N* Ba(OH)<sub>2</sub>.

Two radically different methods have been shown to give almost similar results for the base exchange capacity of a number of soils examined.

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# STUDIES ON TROPICAL SOIL MICROBIOLOGY: I. THE EVOLUTION OF CARBON DIOXIDE FROM THE SOIL AND THE BACTERIAL GROWTH CURVE<sup>1</sup>

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It has long been recognized that the greater part of the carbon dioxide contained in soil air is present as the result of the activities of soil microorganisms: for instance, Smith and Brown (5) have recently found that over 90 per cent of the carbon dioxide produced in samples of Carrington loam was attributable to the presence of microorganisms. During the past few years workers in many laboratories have been engaged in investigations concerned with the liberation of carbon dioxide from the soil, but one of the most important advances in this subject must be attributed to Lemmermann and Wiessmann (4) who, as a result of some experiments in which the daily yield of carbon dioxide from some soil samples was determined, showed that, under the experimental conditions described, the gas evolution was expressed accurately by the equation:

$$x = akt^m \quad \dots\dots(A)$$

where  $x$  is the amount of carbon dioxide formed in time  $t$ ,  $a$  is the initial carbon content of the soil, and  $k$  and  $m$  are constants. This important relationship has been fully confirmed by the writer (1) when working with soils in the Malay Peninsula. Lemmermann and Wiessmann continued their experiments for 3 years: most of the Malayan samples were under test for about 2 months but, in one instance, an experiment was continued for over 6 months.

It is proposed to employ the equation of Lemmermann and Wiessmann in the form:

$$y = Ft^m \quad \dots\dots(B)$$

where  $y$  represents the carbon dioxide produced in time  $t$ , and  $F$  and  $m$  are constants. It must be stressed here that  $x$  in equation (A) and  $y$  in equation (B) both represent the *total* yield of carbon dioxide since the beginning of the experiment; this point is mentioned because a perusal of the literature shows that the difference between the daily and total yields of gas evolved is not

<sup>1</sup> This work was carried out at the Rubber Research Institute of Malaya, and thanks are due to the board of this institute for permission to publish the results.



always appreciated. Examination of the constants<sup>2</sup>  $m$  and  $F$  shows that, while  $m$  is a measure of the falling off in the rate of carbon dioxide production,  $F$  represents the yield of gas in unit time at the beginning of the experiment: if  $t$  be the time in days,  $F$  is the daily yield of carbon dioxide when the experiment is started. Thus, although  $m$  is a factor dependent on the conditions prevailing in the laboratory,  $F$  has an important practical significance.

If attempts are made to assess the microbiological activities of a number of soil samples by evaluation of the carbon dioxide evolved in all cases it is important to determine the factor  $F$  and not merely the value of  $y$ . The factor  $m$  is positive and fractional and hence, under experimental conditions, the rate of gas evolution decreases and, after a period of 3 or 4 weeks, the daily yield of carbon dioxide from a soil sample will be considerably less than it was at the beginning of the experiment. Moreover, after such a period the  $y$  values obtained from a number of samples simultaneously under test may fall into a different sequence from the  $F$  values and so it is imperative to use the latter even for comparative purposes.

As  $F$  represents the yield of carbon dioxide in unit time, it might be supposed that estimations of the gas liberated from soil samples during a short period, say 24 hours, would lead to the direct evaluation of  $F$  but, unfortunately, such is rarely the case. In experiments concerned with the evolution of carbon dioxide from soils in the laboratory there is, almost invariably, an initial period during which the gas evolution is anomalous and not in accordance with the usual equation. The full significance of this period of adjustment, which may last from a day or two to 1 or 2 weeks, has not yet been completely elucidated but it appears that it is curtailed considerably when the soil samples used are fresh and but little disturbed. Nevertheless, the occurrence of a phenomenon of this nature renders the results obtained from experiments of a few hours' or days' duration of somewhat questionable value.

It is, perhaps, hardly necessary to point out that the rate of gas evolution in a soil sample is a function of the temperature, and the equation of Lemmermann and Wiessmann is operative only under thermostatic conditions.

#### THE PRACTICAL IMPORTANCE OF THE RELATIONSHIP $y = Ft^m$

From the agricultural standpoint the value of a soil is largely dependent on the organic matter present and its rate of decomposition, and these factors are included in the rather vague term "fertility." The amount of the organic matter present will depend on the previous history of the soil and also, according to Jenny (3), on its mean annual temperature. A host of factors, such as the size of the soil particles, the aeration of the soil, and its mineral constituents, contribute to the main cause of the decomposition of soil organic matter, namely, the concentration of the microorganisms present, and it seems

<sup>2</sup> These constants can be readily evaluated from the expressions:

$$\log F = \log y - m \log t \text{ and } m = \frac{\log y_2 - \log y_1}{\log t_2 - \log t_1}$$

justifiable to assume that the evolution of carbon dioxide is an index of the activities of these microorganisms. There are many reasons for believing that an accurate evaluation of the rate of gas liberation in the soil would be more pertinent than an estimation of the concentration of microorganisms by the usual plate count method.

In equatorial regions, such as the Malay Peninsula, the temperature at the soil surface in the forest is practically constant; in fact, Haines (2) has shown that the daily variation is only 1°F., and it is unlikely that any serious error is introduced by regarding the soil temperature in more open situations as other than constant; for although the daily variation will be greater than is the case in the forest, the mean daily temperature will hardly change. This being so, and provided that experiments are carried out with fresh soil immediately on its arrival at the laboratory, it is justifiable to assume that the factor  $F$  so

TABLE 1  
*m and F values of soils from the Malay Peninsula*

SAMPLE	ORIGIN	MOISTURE	$m$	$F^*$
		<i>per cent</i>		
A	Primary jungle on the plains	11.6	0.55	19.1
J	Primary jungle at 4,000 feet	34.3	0.73	18.2
K	Primary jungle at 4,000 feet	31.6	0.78	11.2
V	Mangrove forest	36.2	0.85	6.5
L	Rubber estate with the soil under a leguminous cover	32.8	0.63	13.1
P	Rubber estate where a peaty soil had received a dressing of lime some 3 years previously	35.0	0.58	20.7

\*  $F$  represents the daily yield of carbon dioxide in milligrams per 100 gm. of moisture-free soil at the beginning of the experiment.

determined represents the daily yield of carbon dioxide in the field under the equable conditions prevailing in equatorial regions.

In table 1 are given some examples of the  $m$  and  $F$  values found with soils in the Malay Peninsula: there can be no doubt that these  $F$  values give more accurate information regarding the activities of the soil microorganisms than that forthcoming from the usual plate count method. Apart from the generally recognized drawbacks which are inherent in this latter method, its value is further diminished in the tropics by the low concentration of soil microorganisms and the high proportion of fungi over bacteria.

As a result of the extreme seasonal changes which are characteristic of countries situated in temperate regions, the soil temperature varies throughout the year, the concentration of soil microorganisms fluctuates, and the gas evolution varies accordingly. In consequence of these limitations it might appear at first that the estimation of the microbiological activity of a soil by determination of the carbon dioxide liberated under laboratory conditions has little

to commend it. Actually, if experiments were conducted at some arbitrary temperature, determinations of the  $F$  values of a number of soil samples would give information concerning their biological activities which would be relatively correct. If as the experimental temperature were taken the mean value of the soil temperature during the early months of summer, then the results obtained would most nearly approach the actual values for the soil *in situ* during the months when the growth of vegetation and hence the degree of utilization of the products of bacterial metabolism, are at their greatest.

#### THE PHASE OF DECREASE IN THE BACTERIAL GROWTH CURVE

It is well known that when bacteria are subjected to unfavorable conditions, such as those obtaining during the process of disinfection, the decrease in popu-

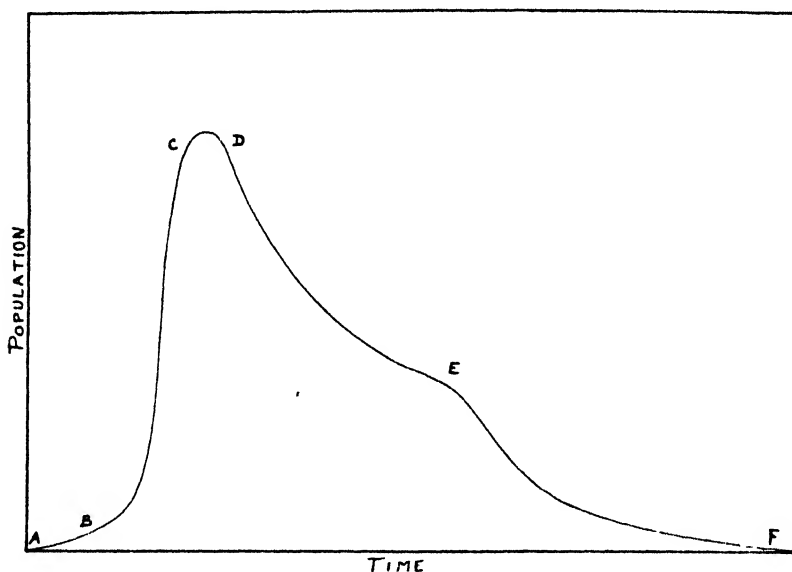


FIG. 1

lation takes place in accord with the exponential law, and the change can be expressed by the relation:

$$-db/dt = kb \quad \dots\dots(C)$$

where  $b$  represents the number of living organisms at time  $t$  and  $k$  is a constant.

Figure 1 illustrates the usual type of curve obtained when the population of a bacterial colony is plotted against the time, and such a curve is characterized by a very rapid increase in numbers for a comparatively short space of time followed by a lengthy period of decline. This curve admits of division into a number of "phases:" the initial *phase of adjustment* ( $A - B$  in figure 1) is followed by the *phase of increase* ( $B - C$ ), and the brief *phase of crisis* ( $C - D$ ) marking a stationary population is succeeded first by the *phase of decrease*

( $D - E$ ) and then by the final *phase of readjustment* ( $E - F$ ). Of these periods, the phases of increase and decrease are by far the most important. Throughout the phase of increase the rate of increase of bacteria at any instant varies directly as the number of bacteria present and may be expressed by the equation:

$$db/dt = kb \quad \dots\dots(D)$$

During the phase of decrease equation ( $C$ ) is followed.

It is evident that the decline in bacterial numbers during the process of disinfection is strictly comparable with the state of affairs obtaining during the phase of decrease in the usual bacterial growth curve. The same is the case with the curves representing the evolution of carbon dioxide from the soil in the laboratory, for here the microorganisms are subjected to an unfavorable environment and gradually die out, although much more slowly than in the presence of toxic vapors. It would be anticipated then that equation ( $C$ ) would hold as is usual throughout the phase of decrease. In the case under consideration, however, the actual number of cells present is not known, but it is found that if the daily yield of carbon dioxide is taken as a measure of the cell population the relation between the daily yield of gas and the time is not exponential. With these soil experiments,  $y$ , the total carbon dioxide evolved between the beginning of the experiment and time  $t$ , is related to the time elapsed by equation ( $B$ ) after a brief initial period as explained in the foregoing.

The soil samples examined contained many different species of bacteria and fungi and it might, with reason, be objected that cases concerned with mixed cultures are hardly comparable with experiments entailing work with pure cultures. Curiously enough it would appear that this objection is not valid. An experiment was carried out with a species of *Actinomyces*, in pure culture, in a glucose solution, and the carbon dioxide evolved was estimated in the same manner as in the soil experiments (1). Direct estimations of the cell population were not made, but, by taking the yield of carbon dioxide in unit time as a measure of the population at any time, it was found that

(a) The curve obtained by plotting the yield of gas in unit time against the time elapsed conformed to the usual type of bacterial growth curve.

(b) During the phase of increase the relationship between the gas yield in unit time and the time elapsed was exponential.

(c) During the phase of decrease this relationship was not exponential, but the total gas yield was related to the time as expressed in equation ( $B$ ).

From the data now in our possession concerning bacterial growth curves, it seems safe to conclude that the results of the carbon dioxide experiments show that

(a) During the phase of increase the rate of evolution of carbon dioxide is an accurate measure of the rate of growth of the population.

(b) In all the soil experiments the microorganisms are passing through the phase of de-

crease and during this period the rate of gas evolution is not directly proportional to the number of viable cells present.

This leads us to suggest that, in the soil, when the microbiological population is increasing, each individual organism plays a part as far as the evolution of carbon dioxide is concerned, and hence a measure of the population is a measure of the biological activity of the organisms. With a declining population, however, some of the organisms are inactive, and determination of the gas evolution is a measure of bacterial activity and not of total numbers present. Thus, when dealing with a declining bacterial population, for practical purposes, a measure of the gas evolution will be of more value than a knowledge of the exact number of microorganisms present.

It seems reasonable to regard the soil of an equatorial country such as that of the Malay Peninsula as being in a stationary condition and at the phase of crisis as far as the population of microorganisms is concerned; and it appears that at this stage the evolution of carbon dioxide will be a measure of the microbiological population present. Even if it were not, for purposes of agricultural interest it would be preferable to evaluate the microbiological activity rather than the total bacterial population.

Smith and Brown (5) have recently described a rapid method of determining the rate of carbon dioxide production in soils under laboratory conditions by means of a modified Barcroft differential manometer and, although their experiments rarely extended over more than 2 days, the writer has found that the results so obtained conform to the expression ( $B$ ) as is the case with the Malayan experiments. The method devised by these investigators promises to overcome the difficulties inherent in the usual method of estimating gas evolution from soils in the laboratory, although it is evident that a period of 2 days is insufficient in which to obtain accurate evaluations of the constants  $F$  and  $m$ .

#### EXPERIMENTAL

The technique employed for the estimation of the carbon dioxide evolution from soil samples was almost identical with that employed by previous investigators, such as Lemmermann and Wiessmann.

The weighed soil sample (about 250 gm.) was placed in a large conical flask connected to a series of absorption tubes containing standard alkali solution; the apparatus was immediately cleaned out by a current of air which had already passed through sulfuric acid, soda-lime, and a 12 per cent (by volume) aqueous solution of sulfuric acid, the last arrangement serving to keep the soil at constant humidity. At regular intervals of from 2 to 4 days the carbon dioxide which had accumulated in the experimental flask was swept into the tubes of alkali by passage of a current of carbon dioxide-free air, and titration was effected with standard acid in the presence of barium chloride and phenolphthalein. As a safeguard against leakage of gas, the alkali tubes were

always replenished and replaced in series with the experimental flask immediately after removal of their contents for titration. It seems probable that a slow continuous current of air passing over the soil throughout the period of the experiment would have been preferable to the practice adopted, but such a procedure was out of the question.

#### SUMMARY

The evolution of carbon dioxide from a soil sample, under laboratory conditions and at constant temperature, is expressed accurately by the relation:

$$y = Ft^m$$

where  $y$  represents the total yield of carbon dioxide after time  $t$  and  $F$  and  $m$  are constants.

Of these constants,  $m$  expresses the retardation in gas evolution due to laboratory conditions while  $F$  is the yield of carbon dioxide in unit time at the beginning of the experiment. This factor  $F$ , therefore, has a practical significance, for it is an accurate measure of the biological activity of the soil *in situ* under thermostatic conditions such as obtain in equatorial regions. Determinations of the  $F$  factors of soils in temperate zones will yield results of value, provided that the experiments are carried out at some arbitrary temperature.

It is shown that, in the laboratory determination of gas evolution from soils, the curve obtained by plotting the daily yield of carbon dioxide against the time elapsed represents the phase of decrease in the general bacterial growth curve and it is deduced that, although the evolution of carbon dioxide is proportional to the numbers of microorganisms present when the population is increasing, during the phase of decrease only a proportion of the living organisms present are concerned in the production of carbon dioxide.

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# A STUDY OF RHIZOBIUM SPECIES IN RELATION TO NODULE FORMATION ON THE ROOTS OF FLORIDA LEGUMES: I<sup>1</sup>

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The presence of nodules on the roots of leguminous plants was first recorded by Malpighi in 1687. He regarded them as root galls. The botanists who studied them during the first half of the nineteenth century considered them a pathological condition. In 1866 Woronin (18), a Russian botanist, found the nodules to be filled with minute bodies resembling bacteria. He also concluded they were abnormal outgrowths due to disease. Later, in 1879, Frank (4) took a forward step. He found nodules on the roots of all healthy legumes. He prevented nodule formation by sterilizing the soil. Hellriegel (9) in 1886, and Hellriegel and Wilfarth (10) in 1888, showed conclusively that nodules on the roots of legumes were due to an outside infection. They proved the real worth of nodules by growing leguminous and non-leguminous plants in sterilized soil of low nitrogen content until such plants were yellow and their development arrested, and then adding a few drops of leachings from unsterilized soils where nodulated leguminous plants were growing. The legumes soon developed nodules and began to flourish, but the non-legumes were not benefited and died. Thus these two German investigators solved a problem of much concern to Boussingault, Laws, and Gilbert and justified an age-old belief of the Roman farmers that leguminous crops enrich the land.

The final and undisputed proof of the relation of bacteria (Rhizobia) to root nodule formation was left to the renowned Dutch bacteriologist, M. W. Beijerinck (1), who, in the year 1888, isolated the causative bacteria in pure culture and caused nodules to develop by artificial inoculation. Schloesing and Laurent (14), in 1890 and 1892, gave the technical proof of nitrogen-fixation by nodule bacteria in symbiosis with legumes by making a quantitative nitrogen determination on seeds and soils, and also on confined air under carefully controlled conditions.

For some years various investigators assumed all legume nodule bacteria to be one species or to be varieties of the same species. In the year 1895, however, Nobbe (25) and his associates found that *Rhizobium* cultures isolated from nodules on the roots of peas (*Pisum sativum*) were unable to produce nodules on the roots of lupines. Hiltner and Stormer (11) in 1903 placed *Rhizobia* from various plant sources in two groups on a basis of flagellation.

The first extensive sound grouping of the organisms working in symbiosis with leguminous plants is probably that of Simon (16), who, in 1914, established five rather definite groups. He conducted cross-inoculation tests with cultures from 34 different species of legumes repre-

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senting 7 genera of the family Leguminosae. A number of strains would infect no species of plants other than the one from which they were isolated. This gave several additional but individual groups. In the same year Garman and Didlake (7) in this country carried out similar cross-inoculation tests which led them to establish six groups. Burrill and Hansen (3), after extensive study of 9 plant genera and 17 species, divided the nodule bacteria into 11 groups, within each of which the host plants are interchangeable. Attention was called to the close botanical relationship of the host plants able to work in symbiosis with the members of a cross-inoculation group of Rhizobia. The group commonly known as the cowpea group is an exception to the rule, as was pointed out. Some of these plants are as different from others as the limits of the large family Leguminosae will permit.

A grouping shown by Walker (17) is, in the main, generally accepted. A great deal of controversy has arisen, however, over the possibility of cross-inoculation among certain plant species. Garman and Didlake (7) reported that Rhizobia responsible for nodules on the roots of the soybean (*Glycine hispida*) were incapable of a symbiotic relation with any other species. Some years later, Leonard (12) working, no doubt, with soybean cultures of a different origin (from a different locality and possibly another variety of soybean), obtained good nodules on the roots of the cowpea. His efforts to inoculate soybeans with various cultures of cowpea bacteria were successful in some instances and of no avail in others. This work has been confirmed (15) and is paralleled in other controversies over inoculation groupings reviewed by Hansen and Tanner (8).

It would seem from Walker's summary that little has been left undone. This, however, is not the case. In Florida and neighboring states many uninvestigated introduced and native leguminous plants bearing root nodules are to be found. Little or nothing is known as to the ability of the bacteria concerned to form nodules and fix nitrogen in symbiosis with various legumes. Also, the underlying cause of the controversies mentioned is important from both a scientific and a practical standpoint.

The work herein reported concerns itself in the main with (a) the ability of a number of the more important of these legumes to work in symbiosis with nodule bacteria from other botanically related plants, and (b)<sup>3</sup> the various properties of the bacteria themselves.

#### CROSS-INOCULATION STUDIES

As has already been stated, the existence of cross-inoculation groups among leguminous plants and their nodule bacteria is generally accepted. Many investigators have sought laboratory methods for identifying representatives of the various inoculation groups. The different legume organisms are so decidedly alike in appearance and behavior on artificial media that morphological and cultural characteristics are of little value in this respect.

Serological methods, which will be taken up in more detail later, were at one time (1912) thought to have promise. Later, however, the agglutination reaction was found to be reliable only when the antiserum for a known nodule producing organism showed a specificity for an unknown.

Nodule production (the so-called Koch's postulates), then, is the only safe criterion for classifying legume bacteria with respect to cross-inoculation groups. The problem of proving an interchangeability with these organisms has two avenues of approach. One is to inoculate seeds of the plant in question with cultures originating from nodules on the roots of host plants whose symbiotic relationship has been established. The other is the reciprocal cross with cultures isolated from the uninvestigated plants on seedlings of proved host plants. This latter test should be preceded by tests on the seeds of the original host plant.

#### EXPERIMENTAL

Cultures representing 14 cross-inoculation groups (established by previous workers) of nodule bacteria, and cultures isolated from the uninvestigated

<sup>3</sup> Section II, to appear in early issue of *Soil Science*.

legumes in question, were used in carrying out the inoculation tests. All cultures were tested on milk and potato media for purity and on seedlings of specific host plants for power of nodule production.

The medium used for the isolation and cultivation of *Rhizobium* cultures was very similar to that recommended by Fred and Waksman (6). It contained the same chemicals, but asparagus extract was substituted for the yeast water. The formula is as follows:

Agar .....	15.0 gm.
Mannitol [ $C_6H_8(OH)_6$ ] .....	10.0 gm.
Dipotassium phosphate ( $K_2HPO_4$ ) .....	0.5 gm.
Magnesium sulfate ( $MgSO_4 \cdot 7H_2O$ ) .....	0.2 gm.
Sodium chloride (NaCl) .....	0.1 gm.
Calcium carbonate ( $CaCO_3$ ) .....	3.0 gm.
Asparagus extract .....	50.0 cc.
Distilled water .....	950.0 cc.

The asparagus extract was prepared by thoroughly macerating the contents of a 10-ounce can of commercial asparagus with approximately 400 cc. of distilled water. This mixture was then pressed through a pad of four thicknesses of cheesecloth and the volume made up to 1,000 cc. with distilled water. This was sterilized in the autoclave in quantities of 50 or 100 cc. and stored in the ice box until needed, the desired amount being added to the agar just before filtering. This medium, without the agar, was employed in the preparation of experimental inocula, but the stock cultures were kept in the solid form.

Cultures representing the various cross-inoculation groups of nodule bacteria grow on this asparagus extract medium quite as well as, if not better than, on the yeast extract agar described by Fred, Peterson, and Davenport (5). The strongest point in its favor is, however, the conservation of the time and effort in its preparation as compared with the preparation of the yeast extract. The procedure (12) for the making of the yeast infusion is as follows:

Dissolve 254 grams of starch-free pressed yeast in 1000 cc. distilled water. Steam for 3 to 4 hours with occasional stirring. Sterilize in deep layers and allow to stand for one week. If undisturbed the yeast cells will settle to the bottom and leave a clear straw-colored liquid above. This clear infusion should be siphoned off, and the reaction adjusted to pH 6.6 to 6.8.

For use, add 100 cc. to the formula given in the foregoing (omitting the asparagus extract, of course).

By way of comparison, cultures from several cross-inoculation groups were planted on the mannitol mineral agar mixture, on the mixture plus yeast water, and on the mixture plus asparagus extract instead of yeast water.

Beijerinck (1) seems to have used extracts from the leaves of peas and beans in gelatin, with sucrose, for obtaining the first pure culture of the organisms. Many investigators have used various legume extracts from leaves and seeds with favorable results. Several such infusions, including alfalfa, red clover, and soybeans, were used by way of comparison in this work. Most of them, without respect to cross-inoculation groups, enhanced the growth to a consider-

able extent. In most cases, however, the plant pigments of these green materials, including green asparagus, discolored the medium, which was a somewhat objectionable feature.

A comparison of several different commercial brands of canned asparagus was made. The conclusion reached was that all brands exhibited about the same effect. The value of the asparagus medium is clearly shown in figures 1 and 2 of plate 1.

The technique used in isolating new cultures of *Rhizobium* is as follows:

Medium sized young firm nodules from the roots of the legume in question were agitated 3 to 4 minutes in a test tube of 70 per cent ethyl alcohol. The nodule was then washed in sterile water or saline and crushed with sterilized forceps in one of four Petri dishes containing approximately 0.3 cc. of sterilized physiological salt solution. Two loops of the milky nodule suspension obtained in the first plate were transferred to a second Petri dish and mixed. The loop was then re-sterilized and the qualitative dilution continued successively to the third and fourth plate. Asparagus extract mannitol agar (approximately 8 cc.) previously cooled to 45°C., was added and thoroughly mixed with the suspension in each of the latter three plates and allowed to solidify. As a precautionary measure against air-borne contaminants, the desk was sponged with 5 per cent phenol, the Petri dishes were left on the phenol-soaked cheesecloth, and currents of air were avoided during the planting process. After 4 to 7 day's incubation at 25° to 28°C., well-isolated colonies were transferred to slants of the agar medium described in the foregoing. The surface colonies usually appear as so many grayish or whitish opaque drops of gelatinous material. The surfaces are usually raised, and the margins entire. The consistency ranges from a slight viscosity to an exceeding ropiness. Color and consistency both vary somewhat within the different groups. *Bacillus radiobacter* is the most bothersome contaminant encountered in the isolation of new cultures. It can usually be recognized by the fact that it grows more profusely and shows more of a watery appearance and consistency than *Rhizobia*. The nodule bacteria were usually kept in refrigeration and transferred once in 4 to 6 weeks when not in use. Stock cultures kept at room temperature were transferred more often.

In the application of the cross-inoculation test the technique was carried out in the greenhouse. The seeds of the legume whose specificity of inoculation was being considered were sterilized, planted, and inoculated in sand maintained in glazed earthenware pots of 1 liter capacity. The sand was of the highest quality pure quartz mined 105 feet below the earth's surface, washed, and ground by the Ford Company for glass making purposes. The pots, which possessed a 10-mm. hole for drainage in the bottom, were filled to within 1 inch of the brim with sand and baked overnight in a hot air oven. Sterilization in this manner was entirely satisfactory. Two methods of sterilization of the seeds were employed. In the earlier part of the work, seeds were treated with 1:1,000 solution of bichloride of mercury ( $\text{HgCl}_2$ ) for 3 to 4 minutes under reduced pressure in a vacuum flask. They were then washed twice with sterile water, once in sterile milk to precipitate any trace of the disinfectant, and once in water to rid them of the milk. Later, immersing the seeds in water at 65°C. for 4 to 5 minutes was found quite sufficient to destroy all nodule bacteria present. This treatment is simple and requires comparatively little time. It was adopted for seed sterilization during the latter part of the work.

The sterilized seeds were planted one-fourth to one-half inch below the surface of the dry sand. In case of the larger seeds, such as peas or beans, 8 or 10 were placed hilum downward with sterilized forceps in each pot and inoculated. The inocula ordinarily used consisted of 5- to 8-day-old cultures of nodule bacteria grown in asparagus extract mannitol solution. Approximately 1 cc. of the culture was allowed each pot. In order to insure early contact between seeds and organisms the culture was added drop by drop in the imprints left by the forceps in placing the seeds.

Sterilized distilled water was then added to bring the moisture content up to approximately 60 per cent saturation. After the moisture content was corrected, the surface of the seedbed was covered with a thin layer of dry sand. This lessened the danger of the various cultures being mixed by visiting insects. Uninoculated controls made up 10 to 15 per cent of each experiment. Two types of controls were maintained for each set of experiments. One type was set up in the usual way, but was not inoculated. Checks of this sort gave an idea as to whether contamination might have occurred. The other type of control consisted of seeds of the host plant inoculated with its known specific bacteria. If good inoculation occurred in this case, it was assumed that conditions were suitable for nodule production. This enabled one to place more emphasis on negative results in the regular test pots.

The tests set up in the manner described in the foregoing were transferred to the greenhouse at this point and kept under observation from 4 to 6 weeks. Wooden strips 1 inch by 1 inch in thickness suspended across the usual greenhouse box-type bench served as supports for the containers, and made it possible to have the openings or drains in the clear, or out of direct contact with any object which might bear contaminants. In addition to this precaution, dust was eliminated as far as possible by wetting down the source of the dust and closing the section to visitors. The plants were visited twice daily and watered when necessary. It might be well to state here that at the time of the first and second waterings, 100 cc. of Bryan's (2) modification of Crone's plant food solution, instead of water, were added to each pot. This mixture contains no nitrogen and has the following formula:

Stock salt mixture:

Potassium chloride (KCl).....	10.0 gm.
Calcium sulfate ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) . . . . .	2.5 gm.
Magnesium sulfate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ).....	2.5 gm.
Tricalcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ).....	2.5 gm.
Ferric phosphate ( $\text{FePO}_4$ ).....	2.5 gm.

Mix all of these salts and grind to a fine powder; use:

Stock salt mixture.....	1.5 gm.
Distilled water.....	1,000.0 cc.

Under the aforementioned treatment the plants developed an abundance of nodules. Certain of the cowpea plants have shown as high as 47 nodules. Contamination has been rare. Only one control out of 49 showed nodule

formation in the work in 1931. No contamination has been evident in 160 tests conducted in 1932.

The results of these cross-inoculation studies are given in summary II, and may be compared with Walker's (17) compilation, summary I.

The name ordinarily used in referring to any one of the cross-inoculation groups is the common name of some representative of the group. In case of the larger group, the common name of the first plant to be investigated is applied to the group. Given by number, they are: I, pea; II, clover; III, alfalfa; IV, lupine; V, cowpea; VI, garden bean; VII, lotus; VIII, soybean; IX, Dalea; and XVIII, black locust.

The groups by the unmentioned numbers, most of which are of a single species, are referred to by the genus names.

In the case of summary II, the groups of plants whose cross-inoculation relationship have been established in these studies are given the corresponding numbers of summary I.

#### SUMMARY I

##### Walker's cross-inoculation group

##### Group I (Pea)

*Lathyrus odoratus*  
*Lathyrus sylvestris*  
*Lathyrus latifolius*  
*Pisum sativum*  
*Pisum sativum arvense*  
*Vicia sativa*  
*Vicia villosa*  
*Vicia angustifolia*  
*Vicia atropurpurea*  
*Vicia daysiicarpa*  
*Vicia faba*  
*Lens esculenta*  
*Cicer arietinum*

*Medicago denticulata*  
*Medicago maculata*  
*Medicago lupulina*  
*Medicago falcata*  
*Medicago hispida*  
*Melilotus alba*  
*Melilotus officinalis*  
*Melilotus indica*  
*Trigonella foenumgraecum*

##### Group IV (Lupine)

*Lupinus perennis*  
*Pupinus luteus*  
*Lupinus angustifolius*  
*Ornithopus sativus*

##### Group II (Clover)

*Trifolium hybridum*  
*Trifolium incarnatum*  
*Trifolium agrarium*  
*Trifolium dubium*  
*Trifolium pratense perenne*  
*Trifolium arvense*  
*Trifolium pratense*  
*Trifolium repens*  
*Trifolium alexandrianum*  
*Trifolium medium*

##### Group V (Cowpea)

*Acacia armata*  
*Acacia floribunda*  
*Acacia linifolia*  
*Acacia longifolia*  
*Acacia melanoxylon*  
*Acacia semperflora*  
*Arachis hypogaea*  
*Baptisia tinctoria*  
*Cajanus indicus*  
*Canavalia ensiformis*  
*Crotalaria retusa*  
*Cyamopsis tetragonoloba*  
*Desmodium canescens*

##### Group III (Alfalfa)

*Medicago sativa*  
*Medicago orbicularis*

SUMMARY I—Concluded

<i>Desmodium illinoense</i>	Group IX (Dalea)
<i>Desmodium purpureum</i>	<i>Dalea alopecuroides</i>
<i>Dolichos lablab</i>	
<i>Dolichos biflorus</i>	Group X
<i>Genista tinctoria</i>	<i>Amphicarpa monoica</i>
<i>Lespedeza striata</i>	
<i>Lespedeza virginica</i>	Group XI
<i>Mucuna utilis</i>	<i>Onobrychis sativa</i>
<i>Phaseolus angularis</i>	
<i>Phaseolus acontifolius</i>	Group XII
<i>Phaseolus aureus</i>	<i>Amorpha canescens</i>
<i>Phaseolus calcearatus</i>	
<i>Phaseolus mungo</i>	Group XIII
<i>Phaseolus acutifolius</i>	<i>Strophostyles helvola</i>
<i>Phaseolus radilus</i>	
<i>Phaseolus luntatus macrocarpus</i>	Group XIV
<i>Pueraria thunbergiana</i>	<i>Crotalaria juncea</i>
<i>Stigolodium deeringianum</i>	
<i>Vigna sinensis</i>	Group XV
<i>Vigna sesquipedalis</i>	<i>Dolichos multiflorus</i>
	Group XVI
Group VI (Garden bean)	<i>Ulex europaeus</i>
<i>Phaseolus angustifolia</i>	
<i>Phaseolus multifolia</i>	Group XVII
<i>Phaseolus vulgaris</i>	<i>Laburnum vulgare</i>
Group VII (Lotus)	Group XVIII
<i>Lotus uliginosus</i>	<i>Robinia pseudoacacia</i>
<i>Lotus corniculatus</i>	
<i>Anthyllis vulneraria</i>	
<i>Tetragonolobus purpurea</i>	
Group VIII (Soybean)	
<i>Glycine hispida</i>	

SUMMARY II

*The cross-inoculation grouping of new host plants*

Group I (Pea)	Group XX
* <i>Lathyrus Tingitanus</i>	<i>Vicia acutifolia</i>
	<i>Vicia Floridana</i>
Group II (Clover)	
* <i>Trifolium carolinianum</i>	Group XXI
<i>Trifolium procumbens</i>	
* <i>Trifolium reflexum</i>	<i>Wistaria Chinensis</i>
Group XIX	Group XXII
<i>Lupinus diffusus</i>	<i>Pithecolobium dulce</i>
<i>Lupinus villosus</i>	

\* Since the completion of this work "Root Nodule Bacteria Leguminous Plants" by Fred, Baldwin, and McCoy has appeared in print giving reference to Pieters' classification, 1927.

## SUMMARY II—Concluded

Group V (Cowpea)	
<i>Apios tuberosa</i>	<i>Crotalaria incana</i>
<i>Aeschynomene americana</i>	<i>Crotalaria intermedia</i>
<i>Clitoria ternatea</i>	<i>Crotalaria juncea</i>
<i>Erythrina herbacea</i>	<i>Crotalaria lancolata</i>
<i>Lespedeza sericea</i>	<i>Crotalaria Maxillaris</i>
<i>Lespedeza stipulacea</i>	<i>Crotalaria mundi</i>
<i>Desmodium triflora</i>	<i>Crotalaria mysorensis</i>
<i>Mimosa strigillosa</i>	<i>Crotalaria oocarpa</i>
<i>Crotalaria alata</i>	<i>Crotalaria polysperma</i>
<i>Crotalaria anagyroides</i>	<i>Crotalaria rotundifolia</i>
<i>Crotalaria axillaris</i>	<i>Crotalaria spectabilis</i>
<i>Crotalaria falcata</i>	<i>Crotalaria striata</i>
<i>Crotalaria goreensis</i>	<i>Crotalaria usaramoensis</i>
<i>Crotalaria Hildebrandtii</i>	<i>Crotalaria valetonii</i>
	<i>Crotalaria vallicola</i>
	<i>Crotalaria verrucosa</i>

In the work from which the data given in summary II were taken, effort was made to test all new cultures on seedlings of the specific host, as was pointed out in the discussion of technique. In two instances, however, this was not done because of difficulty encountered in the obtaining of seeds in one instance and of failure of the seeds to germinate in another. In the case of *Apios tuberosa*, seeds seldom mature, and none could be obtained. Four cultures isolated from different plants of this species gave good nodules on cowpeas (*Vigna sinensis*), however, and this evidence seems sufficient to place it in group V, known as the cowpea group. A somewhat similar situation exists in case of *Mimosa strigillosa*, but again positive results with four isolations seemed to justify placing the species also in group V. In case of *Pithecolobium dulce*, several cultures failed to produce nodules on seedlings of practically all known groups of host plants; consequently this plant, a woody, flowering shrub, was placed in a group by itself. In all other cases the writer is satisfied with the outcome of the tests, since the results of the two-way cross-inoculations made between new and untested species and species of established cross-inoculation groups of host plants were definite and justify the placing given them.

With most of the species placed in the new groups, the decision was based in the main on a failure of the seedlings to develop nodules after being inoculated with cultures from 14 of established cross-inoculation groups. *Vicia Floridan a* and *Wistaria*, both failed to interchange nodule bacteria with any of the old inoculation groups of host plants. The nodules of *Wistaria* reach rather large dimensions, as is shown in figure 3 of plate 1. In the light of newer investigations, the species of host plants given in groups VIII and XIV, *Glycine hispida* and *Crotalaria juncea*, respectively, of Walker's summary, belong in group V, the cowpea group.

The *Crotalaria* species listed in summary II are of special interest. According to McKee and Enlow (13), about 600 species of *Crotalaria* are known. Of this number, however, only 5 species seem to be native to the eastern United States; a majority of the other species being found in the warmer (tropical or

subtropical) portions of other countries. Twenty to thirty species have been introduced by the U. S. Department of Agriculture. Their values as forage and cover crops are now being tested in southern states. Some of the species have promise, while others appear to be worthless. Figure 4 of plate 1 shows one of the more hardy species, probably *Crotalaria spectabilis*, growing in poor Norfolk sand. Inquiries received concerning their cross-inoculation relationships and inoculating material caused effort in this work to be concentrated somewhat upon the available species of host plants of this genus. This accounts for the high percentage of *Crotalaria* shown in summary II of new host plants.

In summary III the cross-inoculation data are brought up to date. The numbering is changed for convenience of arrangement. The common group names are given, however, for convenience in referring to the original summary. Ecological notes on the nativity of many species and genera are tabulated for later consideration. The first column lists legumes by groups in accordance with their cross-inoculation tendencies. In the second column the names of the country from which the plants were first reported are given as far as possible. The third column gives some indication as to temperatures by zones. The fourth column indicates whether the plants are annual, biennial, or perennial.

In the present work, as well as in the literature on similar investigations by others, a great deal of variation with respect to behavior of the Rhizobia of any cross-inoculation group is apparent. As is pointed out elsewhere in this paper, cultures originating from different sources vary from a serological, morphological, and cultural standpoint. In addition, they differ in the power of nodulation and of nitrogen fixation. Most, or all, of these differences or irregularities are generally recognized and accepted among agricultural bacteriologists. Irregularities in nodule-forming power within a cross-inoculation group, however, is not well recognized and is less accepted at the present time. Leonard (12) seems to have been the first to report a variation of this sort. As was stated earlier, several representatives of the cowpea group were able to infect both the cowpea and the soybean. Other similar cultures infected only the cowpea. Controversies over similar cases have been cited.

Inconsistencies of the aforementioned nature have occurred rather frequently during the progress of this problem. It might be stated here, however, that only cowpea and soybean groups of nodule bacteria were considered from this point of view. Twenty-three cultures of the cowpea group of nodule bacteria originating from different sources were tested in duplicate on 10 members of the cowpea group of interchangeable host plants. The data are given in table 1, which shows that certain plants are inoculated by all of the cultures whereas others are infected by only a few. The cowpea plant is well inoculated by all of the cultures, the upper extreme, but the lima bean, generally accepted as a *bona fide* member of the group, is inoculated by cultures from only three of the other host plants in the same group. The peanut presents



SUMMARY III  
The present status of cross-inoculation groups with notes on their origin or nativity

GROUP	COUNTRY	ZONE	HABIT
<b>I (Cowpea group)</b>			
<i>Acacia armata</i> .....	Australia	South temperate	Perennial
<i>Acacia floribunda</i> .....			Perennial
<i>Acacia linifolia</i> .....			Perennial
<i>Acacia longifolia</i> .....	Australia	South temperate	Perennial
<i>Acacia melanoxylon</i> .....			Perennial
<i>Acacia semperflora</i> .....	Southeastern U. S. United States*	North temperate	Perennial
<i>Aschynomene americana</i> .....			Annual
<i>Apios tuberosa</i> .....			Perennial
<i>Arachis hypogaea</i> .....	Brazil	North temperate	Annual
<i>Baptista tinctoria</i> .....	Ky., Fla., Minn.*	Tropical	Perennial
<i>Cojanus indicus</i> .....	India	North temperate	Perennial
<i>Crotalaria karnataka</i> .....	India	Tropical	Perennial
<i>Canavalia ensiformis</i> .....	(Genus widely distributed over tropical and subtropical countries)	Tropical	Perennial
<i>Crotalaria alata</i> .....		Tropical	Annual
<i>Crotalaria anagyroides</i> .....		Tropical	Annual
<i>Crotalaria azillaris</i> .....		Tropical	Annual
<i>Crotalaria falcata</i> .....		Tropical	Annual
<i>Crotalaria gorgensis</i> .....		Tropical	Annual
<i>Crotalaria Hildebrandtii</i> .....		Tropical	Annual
<i>Crotalaria incana</i> .....		Tropical	Annual
<i>Crotalaria intermedia</i> .....		Subtropical	Annual
<i>Crotalaria juncea</i> .....		Tropical	Annual
<i>Crotalaria lanceolata</i> .....	India	Tropical	Annual
<i>Crotalaria masillaris</i> .....			Annual
<i>Crotalaria mundyi</i> .....			Annual
<i>Crotalaria mysorensis</i> .....		Subtropical	Annual

<i>Crotalaria ocarpa</i> .....			Annual
<i>Crotalaria polypetala</i> .....			Annual
<i>Crotalaria retusa</i> .....			Annual
<i>Crotalaria rotundifolia</i> .....			Perennial
<i>Crotalaria spectabilis</i> .....			Annual
<i>Crotalaria striata</i> .....			Annual
<i>Crotalaria usaramensis</i> .....			Annual
<i>Crotalaria vavilovii</i> .....			Annual
<i>Crotalaria vollicola</i> .....			Annual
<i>Crotalaria verrucosa</i> .....			Annual
<i>Cyamopsis tetragonoloba</i> .....			Annual
<i>Desmodium canescens</i> .....			Annual
<i>Desmodium illinoense</i> .....			Annual
<i>Desmodium purpureum</i> .....			Annual
<i>Desmodium triflorum</i> .....			Annual
<i>Dolichos biflorus</i> .....			Perennial
<i>Dolichos lablab</i> .....			Perennial
<i>Erythrina herbacea</i> .....			Perennial
<i>Genista tinctoria</i> .....			Perennial
<i>Glycine hirsuta</i> .....			Annual
<i>Lespedeza stipulacea</i> .....			Annual
<i>Lespedeza sericea</i> .....			Annual
<i>Lespedeza striata</i> .....			Annual
<i>Lespedeza virginica</i> .....			Annual
<i>Mimosa strigillosa</i> .....			Annual
<i>Mucuna utilis</i> .....			Annual
<i>Phaseolus acutifolius</i> .....			Perennial
<i>Phaseolus acutifolius</i> .....			Annual
<i>Phaseolus angularis</i> .....			Annual
<i>Phaseolus aureus</i> .....			Annual
<i>Phaseolus calceolaris</i> .....			Annual

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SUMMARY III—Continued

GROUP	COUNTRY	ZONE	HABIT
<b>I (Cowpea group)—Concluded</b>			
<i>Phaseolus lunatus macrocarpus</i> .....		Tropical to Subtropical	Annual
<i>Phaseolus mungo</i> .....			
<i>Phaseolus radiatus</i> .....			
<i>Pueraria thunbergiana</i> .....	Khasis	Tropical	Perennial
<i>Stigolobium deerlingianum</i> .....			
<i>Vigna sesquipedalis</i> .....		Subtropical	Annual
<i>Vigna sinensis</i> .....	China, Asia	Subtropical	Annual
<b>II (Field pea group)</b>			
<i>Cicer arietinum</i> .....	West Asia	Subtropical	Annual
<i>Lathyrus latifolius</i> .....	Europe	Temperate	Perennial
<i>Lathyrus odoratus</i> .....	Sicily	Temperate	Annual
<i>Lathyrus sylvestris</i> .....	Europe	Temperate	Perennial
<i>Lathyrus tingilanus</i> .....	Africa (Algiers)	South temperate	Annual
<i>Lens esculenta</i> .....	Mediterranean area	Subtropical	Annual
<i>Pisum sativum</i> .....	South Europe	Temperate	Annual
<i>Pisum sativum arvense</i> .....			
<i>Vicia angustifolia</i> .....	Europe	Temperate	Annual
<i>Vicia strobus</i> .....	Algiers	South temperate	Annual
<i>Vicia dasycarpa</i> .....			
<i>Vicia faba</i> .....	North Africa	Subtropical	Annual
<i>Vicia sativa</i> .....	Britain, Eurasia	Temperate	Annual
<i>Viola villosa</i> .....	Eurasia	Temperate	Annual, biennial
<b>III (Clover group)</b>			
<i>Trifolium agrarium</i> .....	U. S., Europe*	Temperate	Annual
<i>Trifolium alexandrinum</i> .....			

<i>Trifolium arvense</i> .....	Europe	Temperate	Annual
<i>Trifolium carolinianum</i> .....	Southern U. S.	Temperate	Perennial
<i>Trifolium dubium</i> .....	Europe	Temperate	Annual
<i>Trifolium hybridum</i> .....	Europe	Temperate	Perennial
<i>Trifolium incarnatum</i> .....	South Europe	Temperate	Annual
<i>Trifolium medium</i> .....	Britain	Temperate	Perennial
<i>Trifolium pratense</i> .....	Britain	Temperate	Perennial
<i>Trifolium pratense perenne</i> .....	Britain	Temperate	Annual
<i>Trifolium procumbens</i> .....	North America	Temperate	Annual
<i>Trifolium reflexum</i> .....	Britain	Temperate	Perennial
<i>Trifolium repens</i> .....	Britain	Temperate	Perennial
IV (Alfalfa group)			
<i>Medicago denticulata</i> .....	Britain, North Africa	Temperate	Perennial
<i>Medicago falcata</i> .....	Europe	Temperate	Annual
<i>Medicago hispida</i> .....	Europe	Temperate	Annual
<i>Medicago lupulina</i> .....			
<i>Medicago maculata</i> .....			
<i>Medicago orbicularis</i> .....			
<i>Medicago sativa</i> .....	Mediterranean area	Temperate	Perennial
<i>Melilotus alba</i> .....	Europe	Temperate	Annual or biennial
<i>Melilotus indica</i> .....	Europe	Temperate	Annual or biennial
<i>Melilotus officinalis</i> .....	Europe	Temperate	Annual or biennial
<i>Trigonella foenum-graecum</i> .....	South France	Temperate	Annual
V (Lupine group)			
<i>Lupinus angustifolius</i> .....			
<i>Lupinus luteus</i> .....			
<i>Lupinus perennis</i> .....	Northern U. S.	South temperate	Annual
<i>Ornithopus sativus</i> .....	Africa, Brazil	Temperate	Perennial
		Tropical	

## SUMMARY III—Concluded

GROUP	COUNTRY	ZONE	HABIT
VI (New group)			
<i>Lupinus diffusus</i> .....		Subtropical	Biennial
<i>Lupinus villosus</i> .....		Subtropical	Biennial
VII (Garden bean group)			
<i>Phaseolus angustifolia</i> .....		Tropical, temperate	Annual
<i>Phaseolus multifolius</i> .....		Tropical, temperate	Annual
<i>Phaseolus vulgaris</i> .....			
VIII (Lotus group)			
<i>Lotus uliginosus</i> .....		Temperate	Perennial
<i>Lotus corniculatus</i> .....	Britain	Temperate	Perennial
<i>Anthyllis vulneraria</i> .....	Britain	Temperate	Perennial
<i>Tetragonolobus purpurea</i> .....	South Europe	Temperate	Annual
IX			
<i>Amphicarpa monoica</i> .....	North America	Temperate	Annual
X			
<i>Dalea alopecuroides</i> .....	U. S.	Temperate	Annual
XI			
<i>Onobrychis sativa</i> .....	Europe	Temperate	Perennial
XII			
<i>Amorpha canescens</i> .....	Missouri	Temperate	Perennial

XIII	Massachusetts, Kansas, Texas	Temperate	Annual
<i>Strophostyles helvola</i> .....			
XIV		Tropical	
<i>Dolichos multiflorus</i> .....			
XV	Western Europe	Temperate	Perennial
<i>Ulex europaeus</i> .....			
XVI	South France	Temperate	Perennial
<i>Loburnum vulgare</i> .....			
XVII	North America	Temperate	Perennial
<i>Robinia pseud-acacia</i> .....			
XVIII	China	Warm temperate	Perennial
<i>Wistaria Chinensis</i> .....			
XIX	Asia, Africa	Tropical	Perennial
<i>Pithecolobium dulce</i> .....			
XX		Warm temperate	Annual
<i>Vicia acutifolia</i> .....		Warm temperate	Annual
<i>Vicia Florida</i> .....			

\* Wide distribution.

TABLE 1  
*Irregularities in nodule formation by root nodule bacteria of the cowpea group tested in duplicate on supposedly interchangeable host plants*

HOST PLANT	SOYBEAN 3	LESPEDEZA 4	COWPEA 10	COWPEA 2	LIMA BEAN	VELVET BEAN	SOYBEAN 121	SOYBEAN 120	SOYBEAN 6	SOYBEAN 5	SOYBEAN 2	SOYBEAN 1
	nodules†	nodules	nodules	nodules	nodules	nodules	nodules	nodules	nodules	nodules	nodules	nodules
Beggar weed.....	—	2 3	5 7	4 7	6 5	7 3	—	—	—	—	—	—
<i>Clitoria tennateca</i> .....	—	—	3 2	4 6	5 4	—	—	—	—	—	—	—
Cowpea.....	11 14	17 13	19 16	31 40	18 15	16 11	7 12	17 14	7 9	5 8	6 7	5 8
Hyacinth bean.....	3 2	4 6	21 27	19 23	16 18	14 29	—	—	Atypical	—	—	—
Jack bean.....	2 1	3 2	3 4	1 3	18 14	—	—	—	—	—	—	—
Lespedeza.....	1 3	4 3	5 4	3 5	1 6	—	—	0 2	2 3	1 3	—	—
Lima bean.....	—	3 5	2 3	14 11	9 10	38 26	—	—	—	—	—	—
Peanut.....	—	Many*	38 21	22 23	0 0	3 2	—	—	Many*	Many*	—	—
Soybean.....	11 12	—	—	—	—	—	6 9	7 6	8 7	4 6	3 4	7 5
Velvet bean.....	—	18 8	2 3	6 4	0 0	11 4	—	—	2 6	3 4	—	—

HOST PLANT	SOYBEAN 13	SOYBEAN 84	SOYBEAN 18	SOYBEAN 41	SOYBEAN 40	COWPEA 28	COWPEA 11	COWPEA 600	COWPEA 8	LESPEDEZA 5	SOYBEAN 915
	nodules	nodules	nodules	nodules	nodules	nodules	nodules	nodules	nodules	nodules	nodules
Beggar weed.....	—	—	—	—	—	6 9	—	3 7	6 6	9 5	—
Cowpea.....	9 11	4 7	9 6	7 6	10 9	10 11	8 12	4 9	7 9	—	8 3
Hyacinth bean.....	7 8	—	—	Many*	3 5	7 6	—	3 8	21 20	3 4	—
Jack bean.....	3 1	—	—	12 14	—	—	—	3 4	4 6	2 3	—
Lespedeza.....	6 5	6 9	4 5	4 3	—	4 6	—	4 5	2 3	11 12	—
Lima bean.....	—	—	—	—	—	26 23	—	14 11	16 12	—	—
Peanut.....	Many*	—	Many*	Several*	Few*	—	Many*	10* 9*	12 20	Many*	—
Soybean.....	14 16	16 9	17 14	9 6	7 8	8 6	4 3	—	6 3	—	7 5

\* Atypical nodules or swellings in the axils of lateral roots. The velvet bean nodule bacteria produced many nodules of this type on the peanut.

† — = no nodules formed; figures = number of nodules in the duplicates of each test; 0 = no test, or that results were thrown out.

another striking fallacy in the policy of group inoculation. In most cases where an infection occurred, the nodules were of the atypical sort which result frequently in cases of incompatible inocula. They occur as small sharply pointed nodules in the axils of secondary and tertiary roots. Inoculation of this type does not seem to benefit the plant.

The irregularities pointed out in table 1 are in keeping, in general, with the other properties of the nodule bacteria, especially those of the cowpea group. The cross-inoculation groups made up of a small number of closely related host plants, such as the alfalfa group comprising 3 genera and 10 species, 8 of which belong to a single genus, and the clover group comprising 10 species of a single genus, present more uniformity from the standpoint of cross-inoculation than is met with in the cowpea group, which was previously made up of 17 genera and 33 species. The addition of new plant species to this group (group V), as given in summary II, brings the grand total, as shown in summary III, up to 21 genera and 63 species.

Conformity of inoculation in so large a group of distantly related legumes is not to be expected. The situation as it exists is paralleled only by the present status of type IV pneumococci which consists of individuals not agglutinated by the immune serum of any other pneumococcus. This, in the mind of the author, explains to a large extent the contradictory results obtained by various workers in this field of endeavor.

#### SUMMARY AND CONCLUSIONS

The methods of isolating and cultivating *Rhizobium* cultures have been simplified by the introduction of asparagus extract as a substitute for the yeast extract which has been in common use for several years.

The cross-inoculation relationships for 41 leguminous species representing 14 genera have been worked out. Thirty-one of these species come within the cowpea group. Twenty-three of the thirty-one additions to this group are species of the genus *Crotalaria*.

Three species all belonging to the genus *Trifolium* were added to the clover cross-inoculation group.

A single species was added to the pea cross-inoculation group.

The *Rhizobia* of six species representing four genera of plants seemed not to be interchangeable with host plants of any of the older cross-inoculation groups; consequently these are considered new groups (see groups XIX, XX, XXI, and XXII of summary II), and are numbered consecutively starting above the last number given in summary I.

The cross-inoculation grouping of two of the individual groups given by Walker in summary I has been changed. The soybean (*Glycine hispida*) and *Crotalaria juncea* have been placed in the cowpea group.

The cross-inoculation data are brought up to date in summary III.

Irregularities in nodule formation have been pointed out in some of the larger cross-inoculation groups, particularly in the cowpea group.

It has been concluded that the present-day conceptions of, and practices in, legume group inoculation are illogical and unsound and should be revised.



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## PLATE 1

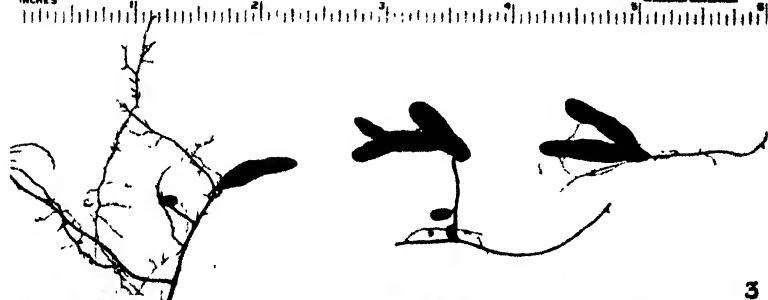
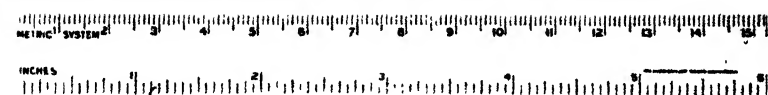
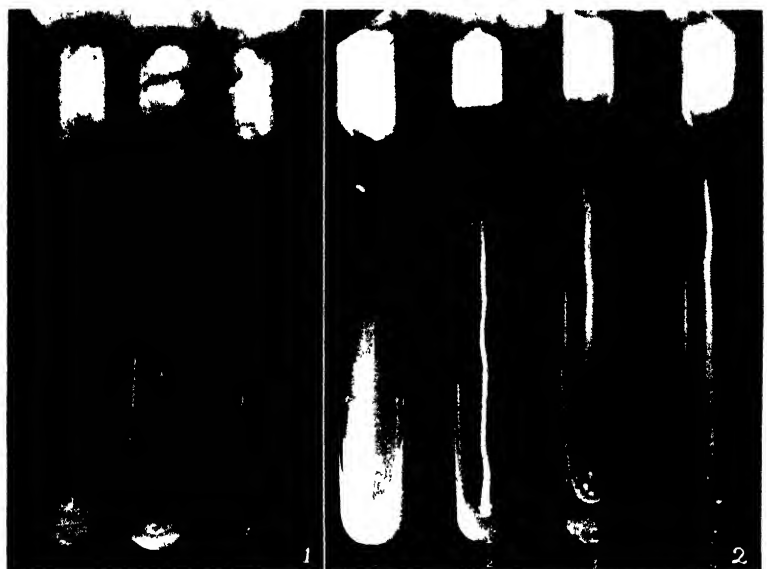
FIG. 1. SOYBEAN CULTURE 6, A SLOWLY GROWING, INEFFICIENT NITROGEN FIXER  
 Tube at left contains no extract.  
 Tube in center contains yeast extract.  
 Tube at right contains asparagus extract.

FIG. 2. SOYBEAN CULTURE 68, A MORE RAPIDLY GROWING, HIGHLY EFFICIENT NITROGEN-FIXING RHIZOBIUM

Tube 1 contains no extract.  
 Tube 2 contains yeast extract.  
 Tubes 3 and 4, duplicates, contain asparagus extract.

FIG. 3. DIMENSIONS OF THE LARGER NODULES OF *Wistaria chinensis*

FIG. 4. A DENSE GROWTH OF *Crotalaria spectabilis*, ONE OF THE HARDIER SPECIES



FIGS. 1 to 4  
135



# STUDIES ON PODZOLS AND BROWN FOREST SOILS: I

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The relationship between the brown forest soils and the podzols has been much discussed since Ramann described the brown forest soils as a special soil type. The purpose of this study is to make a contribution to the characterization of brown forest soils in comparison with typical podzols.

## PODZOLS

A completely developed podzol profile can be described as follows:

*The A<sub>0</sub> layer* consists mainly of organic matter formed by a more or less incomplete decomposition of forest litter or other plant residues. In typical cases it is a duff (20) — raw humus (German "Rohhumus").

*The A<sub>1</sub> horizon* is the top mineral horizon. It contains fairly large amounts of humus and is therefore generally dark colored or gray. In many types of podzols no real A<sub>1</sub> is developed.

*The A<sub>2</sub> horizon* is generally ash-gray or whitish; the humus content is low.

*The B horizon* is sharply separated from A. The color varies from yellowish or reddish brown to very dark brown or nearly black depending on the composition. It is a zone of illuviation where some of the material leached out from the eluvial horizon A accumulates. It is generally more compact than the other horizons and can sometimes form a real hardpan, the so-called "Ortstein."

*The C horizon* represents the parent material, i.e. the original geological deposit which gave the material for the soil formation.

In podzol profiles there sometimes occurs, generally in C or B, a *G horizon*. This is the gley, a hydrogenic formation which develops under the influence of rising and falling ground-water.

The podzol soils and the process of podzolization have been extensively studied by a great number of investigators. Joffe has recently published a review of some of the more important works on the subject of podzolization (6). It is not intended to present here any description of different types of podzols or a classification. Since the soil profiles studied are from Sweden,

<sup>1</sup> The laboratory work involved in this study was done by the author during a stay as Cook-Voorhees' Research Fellow at the New Jersey Agricultural Experiment Station.

The author wishes to express his gratitude to the New Jersey Agricultural Experiment Station and its director, Dr. Jacob G. Lipman, for all the working facilities obtained during the year he worked there as Cook-Voorhees' Research Fellow. The experimental work was done in the laboratory of Dr. Sante Mattson, at that time professor of soil colloids at the experiment station. The author wishes to express his thanks also to Dr. Mattson for many valuable discussions relating to the problems of soil formation.

it may be appropriate to quote descriptions of the Swedish podzol types which are represented.

The Swedish podzols are very well known especially as a result of the investigations of Tamm (23, 28). A general classification of the Scandinavian and Finnish natural soils is given by Frosterus and Tamm (2). Lately Tamm has presented a more detailed classification of the soils in northern Sweden (28).

The podzol profiles later described in this study belong generally to the *iron podzols*. Tamm divides these into three groups because of their variance under different conditions. Those here described agree well with the normal type of iron podzols even if the humus layer sometimes seems to indicate a relationship with a drier type. Tamm describes the normal iron podzol from localities in northern Sweden as follows (28, p. 365):

The humus layer<sup>2</sup> is a duff (raw humus) generally thinner than 10 cm. The A<sub>2</sub> horizon is grayish white with sharp limits. It is somewhat unevenly developed but generally 11–13 cm. thick. The B horizon is rusty red to rusty yellow, sometimes rusty brownish yellow, without sharp limit against the C horizon.

One of the profiles seems to be related to the *iron humus podzols*. This soil type is in all respects a transitional form between the iron podzols and the humus podzols. It is described by Tamm as follows (28, p. 366):

The humus layer is 5–30 cm. thick and consists of a moist duff (raw humus) or peat—there is no sharp limit between these types of humus. The A<sub>2</sub> horizon . . . agrees with the same horizon of the iron podzol but is generally a little thicker. The B horizon is divided in an upper zone B<sub>1</sub>, brownish black about three to four cm. thick, and the underlying B<sub>2</sub>, very much like the B horizon of the iron podzols. . . . A little under B<sub>2</sub> is always found a G horizon.

#### BROWN FOREST SOILS

In the classical works of P. E. Müller are already presented fundamental studies of brown forest soils (15, 16, 17). Ramann made comprehensive investigations of them, studied their regional distribution, and introduced the name "Braunerde," here translated brown forest soil (19). The writer prefers this translation to other terms used because it avoids a confusion with the desert brown soils and gives an idea of the occurrence of the soil type.

The position of the brown forest soils in the soil system has been much discussed. The Russian school of soil science has been inclined to regard the brown forest soil as a subtype of the podzols. Glinka wrote in 1911 (3): "Die westeuropäische 'Braunerde' stellt, so zu sagen, das letzte Stadium des Podsol-(sauren) Typus der Verwitterung dar, der am Wege des Überganges von diesem Typus zum Typus der südlichen Roterden und terra rossa liegt."

Naturally it can be said that it is a matter of opinion where to put the limits between different soil types. But the morphological and chemical features of the brown forest soils are so different from those of the true podzols that they

<sup>2</sup> In Scandinavian terminology the humus layer is called A<sub>1</sub>.

are easily distinguished. And furthermore their relation to a certain type of climate and natural vegetation is evident.

The rather common idea, first expressed by Glinka, that lime carbonate should be necessary for the formation of brown forest soils does not correspond to facts. These soils occur within their climatic region on all kinds of parent material, for instance gravel, sand, and moraines entirely formed of archaean granites and gneisses (26, 27, 30).

An outline of the climatic conditions and the natural vegetation under which brown forest soils develop in Sweden was recently presented by Tamm; he also gave a detailed classification of these soils (27, 30).

A typical brown forest soil profile can be described in the following way: The ground is covered with forest litter under which follow the real soil horizons:

*The A horizon* is a dark homogenous crumb mull (7, 15, 21, 27) of varying depth but generally not more than 10 cm thick.

*The B horizon* is cocoa-brown (like cocoa powder). It is also crumbly, especially in the upper part. There is often no sharp limit between the bottom of A and the top of B. The lower part of B is less crumbly and goes gradually over into

The C horizon, i.e., the parent material.

This is the brown forest soil as described from localities in south-Swedish beech forests. It is developed in about the same way in other forest types of the same climatic region (4, 5, 16, 27, 30). The natural vegetation in this region consists chiefly of hardwood forests, especially beech and oak.

Outside the regional range<sup>3</sup> of the brown forest soils we find in the zone of the true podzols scattered occurrences of soils closely related to the typical brown forest soils. In such cases the development of a profile of the brown forest soil type is due to a parent material containing calcium carbonate (or easily weathered igneous rocks rich in lime) or to a specially favorable site where moving ground-water percolates the soil (4, 8, 24, 27, 30). The natural vegetation type within the podzol region is coniferous forests or mixed birch and coniferous forests. These tree species are also most characteristic of the brown forest soil localities in the podzol region even if some of the hardwood species of the brown forest soil region, though never beech, on such places are found as rarities also in northern Sweden far away from their proper territory.

Because of the different conditions under which they are formed, the climatic ("climatogenic") brown forest soils and more particularly the aclimatic ("lithogenic" or "hydrogenic") brown forest soils vary greatly. For instance: the mull horizon is much thicker in a moist site than in a dry one. As examples, may be taken profiles from different localities at Siljansfors, province Dalarna, middle Sweden (8, p. 92-96). The mull horizon varies here between 2 and 40 cm.; the B horizon is sometimes less than 10, sometimes as deep as 100 cm. This aclimatic brown forest soil is characterized by a high percentage of organic

<sup>3</sup> For information about the regional distribution of podzols and brown forest soils in Sweden see Tamm (25, 26, 30).

matter in the mull horizon, often up to 50 per cent of the dry weight as against a normal content of about 10 per cent in the brown forest soil of the beech forest.

The most striking morphological difference between the brown forest soils and the podzols is the entire absence of a bleached  $A_2$  horizon in the former. The humus-rich uppermost soil layer is developed as a crumb mull in the brown forest soil as against the duff (raw humus) of typical podzols. (There are other types of podzols where a more or less mull-like layer takes the place of the duff.) This means a different type of decomposition of the organic material (17, 20) and hence a somewhat different course of weathering in the soil profile. A possibility of showing by chemical analyses some of the differences in the formation of these soil types is given in the acid-oxalate method.

#### THE ACID-OXALATE METHOD OF TAMM

In trying to find a chemical method of showing the movement of iron in soil profiles Tamm at first used extraction with a saturated aqueous solution of acid potassium oxalate (23). Thus the "limonic" iron in the soil could be determined. Later he changed the acid potassium oxalate to a mixture of ammonium oxalate and oxalic acid in order to get an extract more suitable for the determination, besides iron, of other constituents of the soil colloid complex, primarily aluminum and silica (25). The solution contains, calculated in acid and neutral ammonium oxalate, 0.2 gm. mol of  $(H_4N)HC_2O_4$  and 0.075 gm. mol of  $(H_4N)_2C_2O_4$  per liter; other concentrations tried have been found less suitable. The pH is 3.25. The extraction is done by continuous shaking of 2-5 gm. of soil with 100 cc. of this solution for an hour at room temperature followed by filtering, after which a second extract is prepared in the same way. The combined filtrates and washings are evaporated and gently ignited to volatilize the ammonium salts and burn up the organic matter. Then the ashes are fused with sodium carbonate and analyzed by the common method used for silicate rock analyses. In his last publication on the oxalate method, Tamm recommends a short duration of shaking—half an hour for each extraction—and the use of Membranfilter No. 1 (of the Membranfiltergesellschaft in Göttingen) instead of common filter (29).

#### *Discussion of the method*

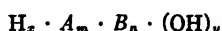
The reaction between the soil colloids and the solution of acid ammonium oxalate is very complicated. The following reactions have to be considered (25, p. 389-390):

The solution acts as an acid.

Base exchange occurs.

The oxalate ions form complex ions with iron, aluminum, manganese, titanium, and other metals.

The work of Mattson has led to the conception of the soil colloids as ampholytoids, amphoteric colloids (12, 13). The general formula of a derived ampholytoid may be written (13):



where  $A$  and  $B$  represent polyvalent weak acids and bases, respectively, with their residual  $H$  and  $OH$  ions. Being an ampholytoid, the compound must be most stable at a certain pH, the isoelectric point. Here the "insoluble" colloid compound will be most difficult to disperse and it will react exchange-neutral against a salt solution of the same pH, provided its ions be "adsorbed" to the same extent by the colloid. But if the salt solution added has a pH below the isoelectric point the compound will give an exchange alkalinity, it will be more dispersible and more soluble.

This last case will apply to the conditions prevailing during the extraction with the oxalate solution. The solution has a pH of about 3.25 and the isoelectric point of the soil colloids is generally higher. As a measure of the isoelectric pH we can take the ultimate pH, the pH of the free ampholytoid after complete electrodialysis. It must lie between the neutral point and the isoelectric pH and in concentrated suspensions approach this point (13). Determinations of the ultimate pH will be reported in the second part of this study. It is sufficient to state here that the ultimate pH of the soils dealt with lies between 3.5 and 6.0. Thus the isoelectric pH should be numerically a little lower. It may further be mentioned that determinations of the point of exchange neutrality of electrodialyzed soils and cataphoresis of the soils confirm these values.

Let us take as an example: What will happen in the case of a colloid built up from iron "hydroxide" and a weak tribasic acid, in itself insoluble? Assuming that one residual  $OH$  ion for each  $H$  ion exists in the isoelectric compound, it may be written in the following formula:  $H \cdot A = Fe \cdot OH$ . The pH of the oxalate solution is assumed to be lower than the isoelectric point of the complex, and the oxalic acid is a stronger acid than the one represented by  $A$  in the formula. Therefore the ion  $H \cdot A = Fe^+$  will predominate, and the dispersibility is increased because the complex has a positive charge. Hydrolytic cleavage of the compound must occur and we will have all kinds of ions down down to the single  $Fe^{+++}$ ; the colloid at the same time changing its composition and at last, if the hydrolysis be complete, forming undissociated  $H_3A$ .

In the case of the soil the conditions are much more complicated. It must be remembered that the radicals  $A$  and  $B$  in the general formula of the colloids are represented by organic compounds, silica, sesquioxides, etc., and the ampholytoid is not free but partly "saturated" with bases. An application of the theoretical discussion will therefore be extremely difficult. Furthermore the adsorbability of the different ions in the oxalate solution is not known with certainty. Anyway, the result of the treatment is a solution containing a part



of the constituents of the colloid and a residue where the colloidal part consists of more acid complexes than the original soil colloids.

The result would be substantially the same if any sufficiently strong acid which forms soluble salts of iron, aluminum, etc., were used. But in the case of the oxalate method we have a buffered solution because of the excess of ammonium oxalate, and then we have to take into consideration the effect of the oxalate ions—the forming of complex easily soluble compounds of the sesquioxides. Therefore the dissolving effect of the acid-ammonium oxalate solution is high. It acts more strongly than an unbuffered strong acid of the same pH. As an example, some figures from Bujakowsky and Treschow may be mentioned. In one soil sample,  $\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3 + \text{SiO}_2$  to the amount of 1.87 per cent of the soil could be extracted with the acid-ammonium oxalate, with 1-N hydrochloric acid only 1.44 per cent was dissolved. In another soil the corresponding values were 6.06 and 4.48 (1, p. 133). Thus the oxalate solution dissolved even more than hydrochloric acid with a numerically much lower pH.

The most interesting question is now: To what extent are iron, aluminum, and silica dissolved from the colloids by the oxalate solution? In order to answer this question a few experiments were made.

Four samples of soil and soil colloids, kindly supplied by Dr. Sante Mattson, were chosen; namely Sharkey colloid, Sassafras colloid, Nipe clay soil, and a soil from New Zealand. The New Zealand soil, obtained through Prof. G. W. Robinson, is characterized by an extremely high percentage of aluminum in the colloidal complex. The three first-mentioned represent widely different types of soils. They have all been previously analyzed in regard to several of their properties (10, 11, 12, 14). The two colloids had been prepared by the supercentrifuge method. The Nipe soil—a true laterite from Oriente, Cuba, containing over 60 per cent  $\text{Fe}_2\text{O}_3$  was electrodialyzed; it consists chiefly of colloidal particles. About 2 gm. of each sample was extracted with acid-ammonium oxalate solution, the extraction being repeated three times, each time with 100 cc. of the solution. Analyses of the extracts gave the results shown in table 1. The figures show that only a comparatively small percentage of the colloids were dissolved by the acid-oxalate solution. There can be several different reasons for the low solubility.

In the case of the Sharkey colloid it could be assumed that the low solubility was due to the low isoelectric point. The figures in the last two columns of the table, quoted from Mattson (12, p. 353), indicate that the isoelectric point should be very near the pH of the oxalate solution. If an acid hydrolysis be the first step in dissolving the colloid, as postulated in the discussion, the solubility should be low: the hydrolysis can not proceed far before the colloid has changed its pH and composition enough to be in equilibrium with the solution.

But this explanation can not account for the low solubility of a colloid like the Nipe. In this case the insolubility is probably due to the inner structure

of the colloid. The laterites are considered as highly mature soils, i.e., the colloids have at least partly undergone processes of "aging." As a general rule the colloids are less soluble when aged than when newly formed. The properties of ferric hydroxide are well known in this respect. Newly precipitated, it is soluble, for instance, in a very dilute hydrochloric acid, but the solubility decreases rapidly with time. If it loses water and becomes iron oxide it will dissolve only slowly even in concentrated acid. The aging of the colloid causes a change in the inner structure rendering it less capable of sending out ions and thus making it less subject to hydrolysis and less soluble. An apparent equilibrium between the colloid and an acid solution may, in such cases, be due to the low reactive power of the colloid. This was illustrated with the Nipe colloid in the following way: A part of the washed residue after the repeated extractions with oxalate solution was put in a flask, new oxalate solution added, the flask closed with a stopper and left undisturbed for some months. During the first days the solution remained colorless but then it

TABLE 1  
*The inorganic compounds extracted with acid-ammonium oxalate*

SOIL OR COLLOID	EXTRACTED INORGANIC SUBSTANCES PER CENT OF DRY WEIGHT				$\frac{\text{SiO}_2}{\text{R}_2\text{O}_3}$		ULTI- MATE pH	pH OF EXCHANGE NEUTRAL- ITY (N NaCl- SOLUTION)
	Total	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	In oxalate extract	In total colloid		
Sharkey colloid . . . . .	4 07	0 74	1 23	1 71	0 54	3 18	3 6	2 9
Sassafras colloid . . . . .	7 86	0 53	3 14	3 71	0 16	1 89	4 7	4 2
Nipe clay soil . . . . .	6 34	0 23	1 09	4 04	0 10	0 31	6 0	6 0
New Zealand soil . . . . .	22 15	6 44	11 95	2 56	0 80			

began to turn yellow, hardly perceptibly at first but increasing all the time until at last the color had become as strong as in the first extract of the colloid. This means that no real equilibrium was obtained during the extractions though the amounts dissolved in the third extract were very small: the colloid continues to send out ions, it hydrolyzes and goes into solution, the iron compounds giving the solution the yellow color.

The solubility, as well as other properties, of natural soil colloids depends to a great extent on their origin. Among the particles of colloidal size there may be fragments of unweathered minerals, as certainly is the case in glacial clay. Such particles are less soluble than those which have been formed by weathering, provided the minerals be of the kinds common in granitic rocks or of similar nature. This has been shown by Tamm in investigations of natural and artificial clays (29).

A comparison between the  $\text{SiO}_2/\text{R}_2\text{O}_3$  ratios in the extracts and in the original colloids is of great interest. It is seen in table 1—where the figures for the total colloid are quoted from Mattson (12, p. 344)—that the ratios are much lower

in the part extracted than in the original colloid. This must mean that the ratio will be wider in the undissolved colloid remaining after the extraction with the acid-ammonium oxalate, which confirms the theory of the dissolving process. This undissolved colloid should have different properties from those of the original soil colloid. For instance, the capacity of cation exchange at a given pH should be increased (10, 11). This was investigated in the following way.

The residue left after the oxalate extractions was washed with a dilute solution of ammonium chloride until the washings showed no oxalate reaction. Then it was leached with neutral *N* barium acetate solution, washed, and the combined Ba displaced by treatment with warm ammonium chloride solution. The combined Ba was determined as BaSO<sub>4</sub>. The cation exchange capacity

TABLE 2

*Cation exchange capacity at pH 7.0 milliequivalents per gram of original colloid or soil and of the residue after extraction with acid-ammonium oxalate*

	ORIGINAL MATERIAL	RESIDUE AFTER EXTRACTION
Sharkey colloid . . . . .	0 74	0 83
Sassafras colloid . . . . .	0 28	0 36
Nipe clay soil . . . . .	0 09	0 08
New Zealand soil . . . . .	0 17	0 26

TABLE 3

*Cation exchange capacity at pH 7.0 milliequivalents per gram of original soil and of the residue after extraction with acid-ammonium oxalate*

	SOIL HORIZON			
	A <sub>2</sub>	B <sub>1</sub>	B <sub>2</sub>	C
Soil . . . . .	0 03	0 11	0 03	0 01
Residue . . . . .	0 03	0 03	0 01	0 01

of the original material was determined in the same way. The results are seen in table 2.

It should be mentioned that the figures in the last column are calculated under the assumption that only inorganic compounds (see table 1) were removed with the oxalate extract, i.e., that the weight of the residue was equal to the original weight minus extracted inorganic compounds. This is not true; organic matter is also dissolved. But this means only that the actual relative increase in exchange capacity probably is a little larger than the one recorded.

The increase in exchange capacity is relatively small in the case of the Sharkey. This might also be expected because the silica/sesquioxide ratio is high in the original complex and fairly high in the part extracted, i.e., the change in the composition of the colloid is comparatively small. In the case of the Sassafras colloid we have a low ratio in the extract and a high ratio in the

original colloid. This means a comparatively high increase in acidoid character of the residue. The relative increase in exchange capacity is high. And where the silica/sesquioxide ratio is low both in the extract and in the original material there is no change in the cation exchange capacity (the actually recorded difference is probably within the limits of the analytical errors).

In some way different results were obtained with samples from a podzol profile (number 5, further described later), as seen from table 3. There is no change in the exchange capacity of the A<sub>2</sub> and C horizons but in the B horizons, especially B<sub>1</sub>, a pronounced decrease occurs. This seems to be contrary to the theory and previously observed facts. The most natural explanation of the results seems to be that the oxalate extraction has removed most of the colloidal material, especially organic matter, capable of taking a place in the base exchange.

### *Conclusions*

The following conclusions can be made on the basis of previously known facts (29) and the results of these experiments:

The products of the chemical weathering in the soil are generally more soluble than unweathered mineral particles, even if they be of colloidal size.

Aging of colloids, formed by chemical weathering, can result in a low solubility.

From these reasons it cannot be expected that the amount of "inorganic colloids" found by the acid-oxalate method should be the same as the amounts found with methods where principally physical properties of the colloids are applied as, for instance, in the supercentrifuge method.

Thus the acid-oxalate method gives a measure of the weathering, not necessarily the total weathering but at least of the unaged products of weathering. The method is therefore very valuable in studying different types of soil formation. Its usefulness in characterizing different soil types has been demonstrated by several authors (7, 18, 25, 28, 29, 31, 32). But it should be pointed out that the method must be used with caution for this purpose in case the soil contains easily soluble unweathered mineral particles (1, 29).

### STUDIES OF PODZOL AND BROWN FOREST SOIL PROFILES

For this study profiles were selected from the provinces of Småland, south Sweden, and Dalarna, middle Sweden. The profiles from Småland were sampled and carefully described by Tamm in connection with his work on Swedish brown forest soils. The author is greatly indebted to Dr. Tamm for the material from these profiles. The profiles from Dalarna were sampled and described by the author during investigations for a description of geology, vegetation, and soils of Siljansfors, an experimental forest of the Swedish Institute of Experimental Forestry, situated about 6 kilometers west of Lake Siljan.

### Description of the profiles

PROFILE 1.—Brown forest soil from Hissön, near Växjö Småland (26, p. 284; 7, p. 15-19; 22 p. 168).

*Vegetation:* Beech-wood (*Fagus silvatica* L.). Hardly any field or ground strata except in small open places in the stand.

*Soil profile:* 2-3 cm. forest litter, mainly beech-leaves.

A. 10-12 cm. crumb mull, brownish black.

B. 40-50 cm. brown soil. The upper part (B<sub>1</sub>) distinctly crumbly, further down less crumbly and of a lighter color. The limits against A and C are not sharp.

C. Sandy-stony moraine. It contains, according to Tamm, granites, leptites, hälleflintas, and small amounts of gneisses, porphyries, diorites, and diabases (7, p. 16).

PROFILE 2.—Podzol from Hollstorp, near Växjö Småland (7, p. 28-30).

*Vegetation:* Mixed pine and spruce wood (*Pinus silvestris* L. and *Picea abies* Karst.). The field strata in the stand are composed of *Calluna vulgaris* Hull (solitary<sup>4</sup>) and *Deschampsia flexuosa* Trin. (infrequent-frequent). The ground stratum is composed of mosses (dominant); most prominent are *Hylocomium parietinum* Lindb. (abundant), *Hylocomium proliferum* Lindb., *Hypnum crista castrensis* L., *Dicranum undulatum* Ehrh.

*Soil profile:* 2-3 cm. forest litter consisting of mosses, pine needles, and spruce needles.

A<sub>0</sub>. 9-11 cm. duff, compact.

A<sub>2</sub>. 4-12 cm. bleached layer, gray (the average depth is 6.5 cm.).

B<sub>1</sub>. 5-10 cm. dark because of the humus content.

B<sub>2</sub>. 20-25 cm. rusty yellow without sharp limit against C.

C. Sandy moraine. It is, according to Tamm, composed of granites, leptites, and small amounts of porphyries, diabases, and diorites (7, p. 28). From a depth of 55. cm. down to 105 cm. under the surface a gley horizon is developed.

PROFILE 3.—Brown forest soil from Siljansfors (8, p. 93-94, profile XII).

*Vegetation:* Spruce-wood of *Anemone hepatica*-type (8, p. 45). Besides the spruce (*Picea abies*) there are also some pine (*Pinus silvestris*), birch (*Betula pubescens* Ehrh.) and low alder trees (*Alnus incana* Moench). The composition of the field strata at the point where the profile was taken is typical of the richest woods of this type within this experimental forest. The dwarf shrubs are abundant but less conspicuous than other species; the most common is *Vaccinium myrtillus* L. Herbs and grasses are abundant; there occur: *Anemone hepatica* L., *Carex digitata* L., *Dryopteris Linnæana* C. Chr., *Eupteris aquilina* Newm., *Fragaria vesca* L., *Melica nutans* L., *Oxalis acetosella* L., *Prunella vulgaris* L., *Veronica chamædrys* L., *Veronica officinalis* L., and several other species. The ground stratum is composed of mosses; the frequency degree is dominant. There are among others *Hylocomium parietinum*, *H. proliferum*, and *H. triquetrum*, the last-mentioned species most characteristic for this wood-type, even if the others generally have a higher frequency (see also 5, p. 429, table 64, No. 10 and 12 from the same stand).

*Soil profile:* 4 cm. forest litter, residues of mosses, spruce needles etc.

A. 8 cm. crumb mull, black, very rich in organic material.

B<sub>1</sub>. 25-30 cm. reddish brown, crumbly mineral soil.

B<sub>2</sub>. 50-60 cm. brown, not crumbly, intensity of color decreasing with increasing depth.

B<sub>3</sub>. 45-50 cm. in the upper part black-brown then with slowly decreasing color, at the bottom light brown.

C. Sandy-stony moraine composed mainly (70-75 per cent) of porphyries—the rocks of the region. Besides the porphyries, sandstones and granites are of some importance. The moraine contains further small amounts of shales, quartzites, and diabases (8, p. 18-22).

<sup>4</sup> The frequency degrees employed in this paper are as follows: dominant, abundant, frequent, infrequent, solitary (9, p. 33).

From another point in the same stand another soil profile was taken (8, p. 93 profile XIV). The mull was here sometimes as thick as 15 cm., but the B horizon was often as thin as 10 cm. This shows the big variation in the development of soil profiles of this type. It is an "aclimatic" brown forest soil formed in a podzol region because of local conditions. The topography causes the water to run more freely than generally, the comparatively high ground-water produces a rich vegetation and the formation of a brown forest soil profile instead of a podzol, though the water is poor in plant nutrients (8, p. 82, table 8).

PROFILE 4.—Iron podzol from Siljansfors (8, p. 87 profile XIII).

*Vegetation:* Hylocomium-rich mixed spruce wood, and pine wood, nearly without field strata but a thick cover of mosses (8, p. 33). The field strata are very sparse; the most common is *Vaccinium myrtillus*. The mosses, mostly *Hylocomium proliferum*, form a thick cover on the ground (see also 5, p. 419–420, table 59, No. 5).

*Soil profile:* 4 cm. forest litter, residues of mosses, spruce needles and pine needles etc.

A<sub>0</sub>. 2 cm. duff, a fibrous F-layer (5, p. 517), hardly any H-layer developed.

A<sub>2</sub>. 8–11 cm. bleached layer, white with a slight pinkish tint, the color probably due to the red porphyry which is one of the most important constituents of the parent material.

B<sub>1</sub>. 10 cm. rusty brownish red, more compact than the following.

B<sub>2</sub>. 60–70 cm. lighter yellowish red, slowly decreasing in intensity of color, without sharp limit against C.

C. Moraine of the same type as in profile 3.

Profiles 3 and 4 were taken only a few meters from each other, profile 4 on a level place a little above the sloping ground of profile 3. Thus profile 4 represents the normal type and 3 a profile developed out of the same parent material under specially favorable conditions.

PROFILE 5.—Iron podzol from Siljansfors (8, p. 87, profile XXIV).

*Vegetation:* Hylocomium-rich mixed pine and spruce wood of *Dryopteris*-type (9, p. 41; 8, p. 35). The predominant tree species is the pine. The little fern *Dryopteris linnaeana* is the most conspicuous species in the field strata; there are further dwarf shrubs: *Vaccinium myrtillus*, *V. vitis idæa* L.; grasses, and herbs: *Deschampsia flexuosa*, *Majanthemum bifolium*, F. W. Schm., *Oxalis acetosella*. Mosses cover the ground, most common are *Hylocomium parietinum* (abundant) and *H. proliferum* (frequent).

*Soil profile:* 2 cm. forest litter formed of mosses, pine needles, etc.

A<sub>0</sub>. 1–2 cm. duff, an F-layer, hardly any H-layer developed.

A<sub>2</sub>. 20–25 cm. pinkish white bleached layer.

B<sub>1</sub>. 15–20 cm. rusty red.

B<sub>2</sub>. 20–25 cm. lighter than B<sub>1</sub>, gradually going over to yellow.

C. Moraine of the same type and composition as in profiles 3 and 4. At a depth of 80–90 cm. under the surface there is a more sandy layer of brownish color. The normal highest level of the ground water seems to be in this layer.

PROFILE 6. Iron podzol from Siljansfors (8, p. 87 profile XXXIX).

*Vegetation:* Lichen-rich pine wood (9, p. 28; 8, p. 25). The pine forms only a sparse tree stratum with open spaces between the trees. The field strata are composed almost entirely of dwarf shrubs; there are hardly any herbs or grasses on the sample plot. Most important among the dwarf shrubs is *Calluna vulgaris* (abundant), next in importance come *Vaccinium vitis idæa* and *V. myrtillus*. The ground is covered with lichens; *Cladonia rangiferina* Hoffm. and *C. silvatica* Rab. are both abundant. Among these there are several other species of little importance.

*Soil profile:* 2 cm. forest litter composed of dead lichens and pine needles.

A<sub>0</sub>. 1 cm. duff, a very loose F-layer.

A<sub>2</sub>. 7 cm. pinkish white bleached layer.

B. 27 cm. reddish yellow, darkest at the top.

C. Moraine of the same type as in profiles 3–5 but a little different composition. The most conspicuous difference is a higher content of sandstones and a corresponding decrease of the granites, these occur here to the extent of only 1 or 2 per cent among the stones as

against an average of 9 or 10 per cent in the area of profiles 3 and 4. Thus the parent material here is very poor in easily weathered material, consisting almost entirely of porphyries and sandstones.

PROFILE 7.—Podzol from Siljansfors (8, p. 87 profile XLVIII).

*Vegetation:* Hylocomium-rich spruce wood of Vaccinium-type (9, p. 36; 8, p. 31). It is a dense spruce wood with a slight intermixture of pine. The field strata consist of abundant *Vaccinium myrtillus* and also, in lower frequency, *Vaccinium vitis idæa* and *Deschampsia flexuosa*; other species are of no importance. The ground stratum is composed of mosses, which form a thick cover; predominant is *Hylocomium proliferum*.

*Soil profile:* 2 cm. forest litter, composed of dead mosses, spruce needles, and so on.

A<sub>0</sub>. 5–6 cm. duff, a compact F-layer on a very thin H-layer.

A<sub>2</sub>. 12–14 cm. bleached layer, a grayish white.

B<sub>1</sub>. 10 cm. very dark brown, sometimes nearly black.

B<sub>2</sub>. 30–35 cm. reddish brown, gradually becoming lighter.

C. Moraine of the same type and composition as in profile 6.

This profile agrees in several respects with the iron humus podzols as described by Tamm. The normal profile type in woods of Vaccinium-type at Siljansfors is an iron podzol; profile 7 is an exceptional case.

### Analyses and discussion

The results of analyses of samples from the different horizons of the profiles previously described are presented in table 4. All analyses were done on samples sieved through a 2-mm. sieve.

The pH was determined in the following way: The air-dried soil samples were shaken with distilled water in a ratio of soil to water of 1:2; in the cases of samples 3A and 6A<sub>0</sub>, in order to get any liquid for the determination, a wider ratio, namely, 1:5 and 1:8, was necessary, because of their great power of absorbing water. After repeated shaking for a day the rubber-stoppered test tubes were left undisturbed for some days and the clear solution was used for colorimetric determinations with a Hellige-Klett comparator and color discs. The indicators used were brom cresol green, brom cresol purple, and brom thymol blue.

Loss on ignition of oven-dry samples is used as a measure of the content of organic matter, a very rough measure but usable for the purpose.

The acid-oxalate method was used in its original form (25), and the results were calculated on the oven-dry basis. The values in table 4 under the heading "other inorganic substances" are calculated as the difference between the total weight of the ignited oxalate extract and the sum of SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, and Fe<sub>2</sub>O<sub>3</sub> determined.

Profiles 1 and 2 were analyzed earlier by the author (7). The analyses are quoted here as standards for comparison with the new material, recalculated on the oven-dry basis instead of, as previously, on the mineral basis.

The differences between the various soil types are clearly seen in the results of these analyses. Let us first look at the typical brown forest soil (profile 1):

The pH is nearly the same (about 6) in all horizons.

The loss on ignition (for exact values of content of organic matter see 7, p. 18 table 2) is high in the whole A horizon, lower and decreasing with increasing depth in the B horizon, and still lower in the C horizon.

TABLE 4  
*Analyses of podzol and brown forest soil profiles*

PROFILE NUMBER	HORIZON	pH	LOSS ON IGNITION	EXTRACTED WITH ACID-AMMONIUM OXALATE (TAMM'S METHOD)			
				SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	Other inorganic substances
			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	A	6.1	10.52	0.02	0.47	0.55	0.30
	B <sub>1</sub>	5.7	4.91	0.06	0.40	0.59	
	B <sub>2</sub>	5.9	2.54	0.08	0.57	0.51	0.22
	C <sub>1</sub>	6.1	1.19	0.07	0.37	0.36	0.15
	C <sub>2</sub>	5.9	1.73	0.07	0.36	0.15	0.07
2	A <sub>2</sub>	4.0	6.16	0.06	0.46	0.38	
	B <sub>1</sub>	4.7	7.58	0.48	2.18	0.91	
	B <sub>2</sub>	4.9	5.42	0.60	2.22	0.61	
	C <sub>1</sub>	5.3	2.45	0.42	1.39	0.27	
	G		1.40	0.11	0.68	1.34	
	C <sub>2</sub>	6.2	0.50	0.12	0.19	0.31	
3	A	4.8	46.28	0.20	2.52	3.67	1.10
	B <sub>1</sub>	6.1	3.79	0.59	1.02	2.24	0.49
	B <sub>2</sub>	6.2	1.85	0.07	0.36	0.70	0.04
	B <sub>3</sub>	6.2	2.23	0.03	0.41	0.86	0.12
	C	6.2	1.29	0.05	0.20	0.37	0.06
4	A <sub>2</sub>	4.1	1.62	0.07	0.11	0.04	Trace
	B <sub>1</sub>	6.1	3.52	0.56	2.33	0.93	0.17
	B <sub>2</sub>	6.1	1.82	0.17	0.67	0.56	0.10
	C	6.1	1.32	0.09	0.23	0.22	0.10
5	A <sub>2</sub>	4.5	1.34	0.04	0.10	0.04	0.08
	B <sub>1</sub>	5.8	6.31	0.61	2.22	0.97	0.35
	B <sub>2</sub>	6.1	2.15	0.30	0.87	0.28	0.04
	C <sub>1</sub>	6.2	1.27	0.13	0.30	0.18	0.09
	C <sub>2</sub>	6.2	1.49	0.13	0.39	0.18	0.11
	C <sub>3</sub>	6.2	1.37	0.18	0.49	0.08	0.01
6	A <sub>0</sub>	3.8	90.71	0.03	0.35	0.11	0.18
	A <sub>2</sub>	4.1	2.32	0.03	0.15	0.07	0.01
	B	6.1	4.86	1.37	3.64	1.05	0.14
	C	5.9	0.68	0.09	0.26	0.10	0.01
7	A <sub>2</sub>	4.7	2.25	0.04	0.08	0.06	0.09
	B <sub>1</sub>	5.7	11.66	0.92	3.70	1.43	0.28
	B <sub>2</sub>	5.7	10.12	0.33	2.82	4.43	0.17
	C	5.9	1.32	0.10	0.32	0.19	0.10



The acid-oxalate extracts show a low amount of silica throughout the profile but lowest in the A horizon. The sesquioxides are fairly constant in the A and B horizons and lower in C. Other inorganic substances decrease from A to B and C.

Some of these results are graphically illustrated in figure 1, the graphs are drawn in the way proposed by Tamm (28), which is undoubtedly better than the one previously used by the author (7, 22).

The "acclimatic" brown forest soil (profile 3) shows some differences:

The pH is rather low, 4.8, in the A horizon. This value is fairly characteristic. Hesselman found a pH of 5.0 as an average for the mull in woods of the *Anemone hepatica*-type at

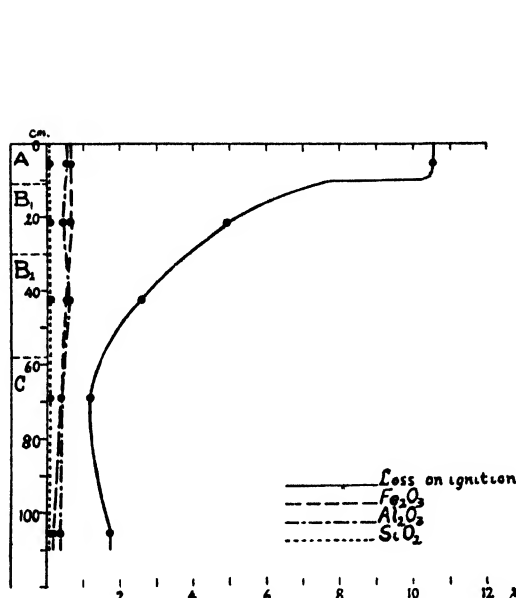


FIG. 1. THE LOSS ON IGNITION AND THE AMOUNTS OF SILICA AND SESQUIOXIDES EXTRACTED WITH THE ACID-OXALATE METHOD, PER CENT OF OVEN-DRY SOIL, IN PROFILE 1

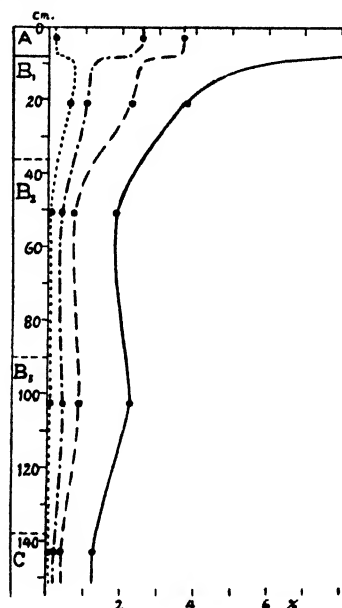


FIG. 2. THE LOSS ON IGNITION AND THE AMOUNTS OF SILICA AND SESQUIOXIDES EXTRACTED WITH THE ACID-OXALATE METHOD, PER CENT OF OVEN-DRY SOIL, IN PROFILE 3

Siljansfors (5, p. 224, 519). In the B and C horizons the pH is above 6 without any clear difference between the horizons.

The loss on ignition is very much higher in the A horizon, nearly 50 per cent as against a little more than 10 per cent in profile 1. In the B and C horizons there is no difference between the profiles in this respect: the loss on ignition is fairly low and decreasing in the B horizon and low in C.

The acid-oxalate extracts show a maximum of silica in B<sub>1</sub>. It is lower in A but much higher there than in the lower B horizons and C. The sesquioxides have their maximum in A and decrease through the B horizons down to the low values in C. The same is the case with "other inorganic substances."

A graphical illustration is given in figure 2.

During the analyses it was observed that the acid-oxalate extracts of the A and B<sub>1</sub> horizons contained rather large amounts of manganese. Generally the content of this element has been very low in the oxalate extracts of samples from podzol and brown forest soils analyzed by the author. But in this case it seemed worth while to determine it quantitatively. For comparison manganese was determined in all the horizons of the profile and in the podzol profile number 4. The results are seen in table 5. The manganese is distributed in this brown forest soil profile in the same way as are the sesquioxides, it occurs only in smaller amounts. The podzol profile shows the common low percentage of manganese, but even in this case there is a resemblance in the occurrence of manganese and the sesquioxides, i.e., the maximum is in the B horizon, which is characteristic for the podzols.

The difference in the distribution of the sesquioxides in profiles 1 and 3 would be less conspicuous if the values were calculated on the volume basis instead of the weight basis. This method of calculation would give a truer picture of the concentration of this element. Romell has recently pointed out the conse-

TABLE 5  
*Acid-oxalate soluble manganese MnO per cent oven-dry material*

PROFILE NUMBER	SOIL HORIZONS				
	A	B <sub>1</sub>	B	B <sub>2</sub>	C
3	0 80	0 14	0 02	0 02	0 02
4	Trace	0 02	0 01		Trace

quences of calculating the humus content of mull and duff profiles in this way (20). In the present case the result would be a comparatively lower amount of sesquioxides in A of profile 3 because of the low volume weight. But in any case there would remain some clear chemical differences between these two types of brown forest soils, for instance the high amount of oxalate-soluble compounds in the last type and the enriching of silica in the B horizon.

*The typical iron podzol* (profiles 4, 5, 6) is entirely different from the brown forest soils:

The pH is very low, 4.1-4.5 in the A<sub>2</sub> horizon and higher, about 5.8-6.2 in B and C. In the case where an A<sub>0</sub> was analyzed the pH was lowest in this horizon, as is generally the case in podzol profiles with duff.

The loss on ignition is low in the A<sub>2</sub> horizon, fairly high in B<sub>1</sub>, decreasing in the lower part of B, and is still lower in C.

The acid-oxalate extracts show very small amounts of silica and sesquioxides in the A<sub>2</sub> horizon. The B horizon, especially its upper part, is rich in these compounds. They decrease gradually in the lower part of B. In C the amounts are low but always higher than in A<sub>2</sub>. Other inorganic compounds also have a clear maximum in the upper part of the B horizon. The A<sub>0</sub> sample analyzed (profile 6) contains amounts of silica and sesquioxides comparable with the A<sub>2</sub> and C horizons. But it must be pointed out that if the values were calculated on the mineral basis the results would be entirely different because of the high content of

organic matter in  $A_0$ . In fact, the sesquioxides will be very near the same in  $A_0$  and B. If, on the other hand, the calculation were made on the volume basis the amounts of silica and sesquioxides would be very much lower in  $A_0$  than in any other horizon: the "concentration" of these colloidal elements is lowest in this horizon.

Graphical illustrations are seen in figures 3 and 4.

Profile 2 differs from the other podzol profiles by having a rather high content of organic matter in the  $A_2$  horizon. The distribution of the other constituents determined is the same as in the typical iron podzols. The G horizon has a high content of oxalate-soluble iron, which is characteristic for such horizons (28, p. 293; 392).

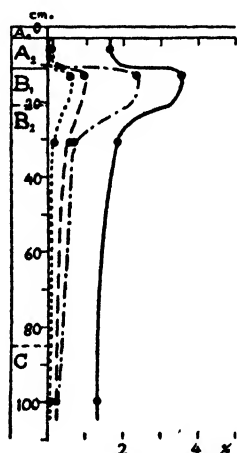


FIG. 3. THE LOSS ON IGNITION AND THE AMOUNTS OF SILICA AND SESQUIOXIDES EXTRACTED WITH THE ACID-OXALATE METHOD, PER CENT OF OVEN-DRY SOIL, IN PROFILE 4

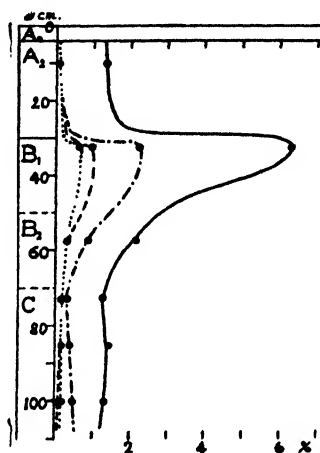


FIG. 4. THE LOSS ON IGNITION AND THE AMOUNTS OF SILICA AND SESQUIOXIDES EXTRACTED WITH THE ACID-OXALATE METHOD, PER CENT OF OVEN-DRY SOIL, IN PROFILE 5

(For legend, see figure 1)

Profile 7 agrees in some way with Tamm's iron humus podzol type (28, p. 198; 366). The most characteristic features are the high amounts of humus and oxalate-soluble silica and sesquioxides in the B horizons. A difference between this profile and the type profiles described by Tamm is the distribution of aluminum and iron: aluminum has here its maximum in  $B_1$  and iron in  $B_2$  as against the opposite in the typical iron humus podzol. A graphical representation, figure 5, compared with figures 3 and 4 gives a good illustration of the differences between this profile and the typical iron podzols.

The results of the investigation may be summarized as follows:

The morphological differences between podzols and brown forest soils correspond to chemical differences which can be analytically demonstrated with the acid-oxalate method. In the podzols we find a minimum of oxalate-soluble iron, aluminum, and silica in the bleached

horizon,  $A_2$ , and a marked maximum in the B horizon. In the brown forest soils there is no horizon corresponding to  $A_2$  and no minimum of oxalate-soluble compounds in the upper soil horizons. The sesquioxides, especially, occur in about the same amount in the A and B horizons; if there is a maximum, as in the case of the "acclimatic" brown forest soil, it occurs in the mull, the A horizon.

The pH values of the horizons indicate that the differences in the formation of the two soil types can be correlated to a different type of weathering. In the case of the podzols the sesquioxide compounds formed by the weathering are washed out from the upper horizons because of the acidity; they are precipitated in the horizon where the pH is high enough to correspond to their isoelectric points or at least their range of greater stability. In the brown forest soil the prevailing pH is high enough even in the uppermost horizon to prevent the sesquioxides from remaining in the sol stage; they are precipitated in both the A and the B horizons.

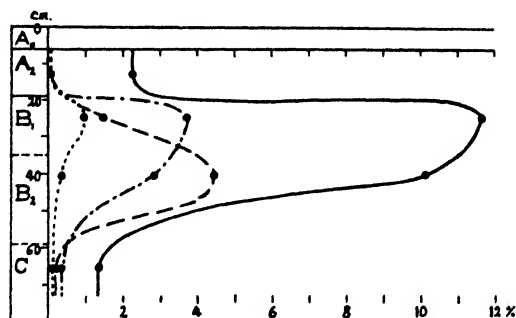


FIG. 5. THE LOSS ON IGNITION AND THE AMOUNTS OF SILICA AND SESQUIOXIDES EXTRACTED WITH THE ACID-OXALATE METHOD, PER CENT OF OVEN-DRY SOIL, IN PROFILE 7  
(For legend, see figure 1)

In a second part of this paper will be reported experiments dealing with electrodialysis, ultimate pH, exchange neutrality, cataphoresis, cation exchange capacity, and absorption of acid and basic dyes, carried on with the same soils. The results will permit a more detailed presentation of the chemical differences between the podzols and the brown forest soils.

#### SUMMARY

A description of the podzol and brown forest soil types is given with special references to some of the subtypes which occur in Sweden.

The acid-oxalate method of Tamm is presented and discussed together with some experiments made in order to illustrate its applicability. The method gives a measure of the weathering and is useful in characterizing different soil types.

The soil profiles further investigated are described together with some notes on vegetation and geology of the sample plots.

It is shown that the oxalate method is very good as a means of chemically characterizing the differences in the formation of these podzols and brown forest soils. The relationship and the differences between the "climatic" and the "acclimatic" brown forest soils are demonstrated.

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# THE NATURE OF SLICK SOIL IN SOUTHERN IDAHO<sup>1</sup>

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In the southwestern part of Idaho there are spotted areas in the soil that hardly maintain plant growth. Kelley and Brown (17) and Peterson (22) have called attention to these areas. They are commonly known as "slick spots" from the fact that they will not let water pass through and, when wet, give a shiny appearance in contrast to the surrounding soils, which give a normal appearance of a soil when moist.

The terms "slick" and "normal" soil will be used throughout this paper to designate this abnormal soil and the normal soil surrounding it.

Ordinarily these slick spots are from a few to several hundred square yards in size and are promiscuously scattered in a field. The location of these spots has no particular reference to the topography of the land, and the only way they can be recognized is by the land being void of any vegetation, which is mostly sagebrush. In a cultivated field they may be recognized by the poor physical condition of the soil when dry, or the aforementioned shiny appearance when wet, and by deficient plant growth if crops have been planted.

Soil profiles were examined on a number of these spots and in the adjacent normal soils. This study brought out one thing: Below both the slick, and normal soils there is a rather heavily impregnated calcareous layer which occurs at varying depths, ranging from 5 to 15 inches below the surface. The calcareous layer may vary in depth, but normally extends several feet below the surface. Much of this layer is underlaid by a caliche layer; very commonly there is in addition a layer of coarse gravel, the shape of which shows that the soil is water deposited.

## PURPOSE OF THE EXPERIMENT

Since the slick spots at times comprise an area from 10 to 40 per cent of the total in a field, their reclamation becomes of great economic importance, and ways and means have been sought to improve them with the lowest expenditure. In order to find some of the fundamental differences that exist between the slick and the normal soils, and to see how different admixtures of these soils may affect each other, a set of samples was made up to serve as a basis for study of the plant relationship to the physicochemical make-up of these soils.

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## PREPARATION OF SAMPLES

A set of 10 bulk samples was taken at the Caldwell Substation in the fall of 1930. They were taken at a depth of 7 inches in the desert, and each slick spot sample had a corresponding normal sample, taken as close as possible to the slick spot but in a place where the vegetation was normal. The samples taken were large enough to permit carrying on of greenhouse work and were shipped to the experiment station laboratory at Moscow, where all the work was done. A series of 11 samples, numbers 10234 to 10244, was then prepared, using the proportions of slick to normal soil ranging from 10 to 0 in the first to 0 to 10 in the last.

The main portion of each was put in 4-gallon jars, 40 pounds to a pot, for greenhouse studies, and subsamples of each were taken for studies in the laboratory.

TABLE 1  
*Chemical analyses on slick and normal soils from Caldwell Substation*

NUMBER	DESCRIPTION		SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	P <sub>2</sub> O <sub>5</sub>	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	LOSS IN IGNITION	N
			per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
18	Surface	Slick	65.62	14.70	4.49	0.11	3.82	1.47	2.42	2.31	1.67	0.06
20	Surface	Normal	67.97	14.05	4.52	0.25	3.45	2.33	2.63	2.51	2.20	0.07
22	Surface	Slick	63.28	14.78	3.17	0.23	3.90	3.64	2.48	1.39	3.88	0.05
24	Surface	Normal	69.70	15.34	3.48	0.18	2.91	1.74	2.37	2.36	3.89	0.08
41	Surface	Slick	63.10	15.66	5.07	0.15	2.04	1.70	1.62	2.55	4.52	0.11
42	Surface	Normal	62.40	15.43	5.90	0.18	2.59	1.34	2.56	2.96	5.25	0.01
1	Surface	Slick	67.22	16.15	4.65	0.12	2.99	1.33	2.06	2.81	3.37	0.04
2	Surface	Normal	70.15	15.79	4.31	0.11	1.79	1.11	2.22	2.54	3.03	0.06

It was felt that this was a safe procedure because chemical analyses obtained from the work of Peterson (22) on samples from the same locality (samples 18 to 42 in table 1) and analyses of samples 1 and 2 made in connection with some other work (unpublished data) show that the chemical composition of these soils varied very little. The pH determinations, too, made on the original samples at the time they were taken, did not show great differences within each group.

## RESULTS OF GREENHOUSE STUDIES

The soil samples in pots were planted to corn, 10 seeds to each pot, and after the plants were about 1 inch high they were thinned down to three plants in a pot. Each soil was run in triplicate. Records were kept of all moisture added. In the pots where the soil was predominantly slick the moisture was taken up very slowly, and when dry the surface was full of cracks. The size of the cracks decreased as the proportion of the normal soil increased over that of the slick soil. There is no doubt that the difference in the cracks is due to

the different amounts of colloidal material present in each soil; mechanical analysis of these soils, as will be shown later, proved that to be the case.

Additional water was added to the pots only after the plants commenced to wilt. This was done for the purpose of using only the minimum amount of water to produce the maximum plant growth. The pots with the slick soil or mostly slick soil usually required water at least a week ahead of the pots with the normal soil.

After the corn had tasseled, it was cut even with the ground and oven dried. The amount of water actually used in the process of growing was computed and the amount of water used to produce 1 gm. of dry matter was calculated. The results and also the moisture content of the soils after the completion of the experiment are shown in table 2.

TABLE 2  
*Data of greenhouse work*

SOIL NUMBER	SUM OF THREE POTS		AMOUNT OF H <sub>2</sub> O USED TO PRODUCE 1 GM. OF DRY MATTER	MOISTURE CONTENT OF SOIL AT TIME OF HARVEST— AVERAGE OF THREE POTS
	Amount of H <sub>2</sub> O used during growth	Weight of dry matter produced		
	<i>cc.</i>	<i>gm.</i>	<i>cc.</i>	<i>per cent</i>
10234*	9,214	4.42	2,085	10.7
10235	13,400	6.78	1,990	11.3
10236	13,317	7.23	1,845	11.4
10237	13,598	13.11	1,035	10.8
10238	14,082	18.92	745	9.7
10239	14,380	21.06	683	8.7
10240	14,695	24.58	597	8.3
10241	15,151	19.46	775	6.9
10242	15,330	20.48	747	6.5
10243	16,063	20.12	798	5.2
10244	15,971	22.48	709	5.3

\* Run in duplicate only.

There is a distinct gradation in the amount of water used to produce 1 gm. of dry matter for the different soils. Samples 10239 and 10240, 50 and 40 per cent slick soils, respectively, show a small difference from the regular trend. This probably was due to a slightly lighter packing of the soil when planted, which resulted in the plants getting a better start at the beginning of the experiment.

Two factors are responsible for the uneven utilization of water: first, there is a higher evaporation of water from the surface of the pot in the slick soil, and secondly, there is an actual difference in yield in favor of the normal soils. These two points will be discussed later.

#### RESULTS OF LABORATORY STUDIES

High alkalinity of soils is considered one possible cause of deficient plant growth in irrigated sections. That this is not the case with slick soils is shown

by the fact that all the soils used in this work had comparatively low pH values and contained relatively small amounts of water-soluble salts.

The pH determinations are reported in the last column of table 3; the hydrogen electrode being used for the determinations. Water-soluble salts were

TABLE 3  
*Exchangeable bases and pH in soils*

SOIL NUMBER	Al	Ca	Mg	Na	WATER-SOLUBLE Na m.e. per 100 gm. of soil	RATIO OF EXCHANGEABLE Ca.Na	pH OF SOIL
	m e. of exchangeable bases per 100 gm. of soil						
10234	0 89	9 83	3 14	5 75	6.22	1.71	7 99
10235	0 50	9.53	3.09	4 95	5 73	1 93	7.89
10236	0 50	9 41	3 01	4.71	5.01	2.00	7.93
10237	0.38	9 27	2 75	3 77	4.58	2.46	7 86
10238	0 38	8.92	2.54	3 52	4.00	2.54	7 69
10239	0.38	8.34	2.46	3 08	3.30	2.71	7 52
10240	0.30	8.34	2.30	2 09	3 10	3.99	7 52
10241	0.30	7.50	2.12	1 52	2.44	4.93	7.53
10242	0.21	7.62	1 94	1.20	1.66	6 35	7.52
10243	0.21	7.50	1 76	0 46	1.27	16.30	7.53
10244	0.21	7.26	1.62	0 15	0 48	48.40	7.30

TABLE 4  
*Mechanical analysis of soils*

SOIL NUMBER	, DIAMETER OF PARTICLES IN MILLIMETERS							
	2 0-1.0	1 0-0.5	0.5-0.25	0.25-0.10	0.10-0.05	0.05-0.005	0.005-0.002	Colloid <0.002
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
10234	0.49	0.52	1.59	6.57	12.16	50.01	6.22	23.40
10235	0.29	0.86	1.78	6.95	13.75	49.50	6.01	21.80
10236	0.42	1.08	1.88	7.08	14.87	49.25	5.83	20.50
10237	0.44	1.13	2.15	7.25	15.92	50.30	5.07	18.68
10238	0.37	1.18	2.24	8.12	16.07	51.10	5.38	16.65
10239	0.47	1.00	2.20	7.98	17.00	50.70	5.13	15.95
10240	0.43	1.25	2.26	8.17	18.62	50.50	4.90	14.09
10241	0.45	1.15	2.46	9.13	19.28	50.57	4.86	12.96
10242	0.38	1.27	2.58	9.18	19.81	50.20	5.22	11.32
10243	0.39	1.38	2.53	9.96	22.03	48.80	5.02	10.11
10244	0.44	1.51	2.76	9.94	22.35	50.70	4.75	8.57

run in connection with the determination of the exchangeable bases, and only soluble Na was found; the amounts are given in table 3.

It is felt that the low hydrogen-ion concentration is due to a low water-soluble salt content. Breazeale and McGeorge (5) and Kelley and Brown (17) mention a low water-soluble salt content of slick soils. They do not indicate the pH of these soils, however.

It has been recognized by many soil investigators [Anderson (1), Gedroiz (9, 11, 12), Cummins and Kelley (7), Hissink (14), Kelley and Brown (17)] that the exchangeable Ca and Na have a profound influence on the properties of the soil. Determinations of exchangeable bases of the soils studied were first made according to the HCl method of Gedroiz (10) as used by Puri (24), but the fact that this method gives large quantities of aluminum and iron in the extract makes it a very doubtful method. For that reason the ammonium acetate method of Schollenberger (25) was finally adopted. The results are given in table 3.

The ammonium acetate extract showed only traces of K in the first three samples. No exchangeable K was found in the remaining soil samples.

As a further study the mechanical analysis of these soils was made. The determination was made according to the method of Olmstead, Alexander, and Middleton (21). The results are given in table 4.

TABLE 5  
*Dispersion factor and dispersion coefficient determinations*

SOIL NUMBER	D. F.	COLLOIDAL CLAY	D. C.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
10234	4.45	23.40	19.00
10235	4.66	21.80	21.35
10236	4.19	20.50	20.50
10237	3.72	18.68	19.90
10238	3.46	16.65	20.80
10239	3.66	15.95	22.90
10240	3.53	14.09	25.05
10241	3.42	12.96	25.40
10242	3.27	11.32	28.85
10243	3.39	10.11	33.40
10244	3.05	8.57	35.70

At the same time, two factors proposed by Puri (23) to determine the physical state of a soil, the dispersion factor (D. F.) and dispersion coefficient (D. C.), were determined. The results are shown in table 5.

#### DISCUSSION OF RESULTS

It was stated in the beginning of this paper that no actual chemical analyses were made on the samples used in this work, but analyses taken from Peterson's work and chemical analyses made in connection with other work (unpublished data) show only significant differences in the  $\text{SiO}_2$  content if samples 41 and 42 are disregarded (table 1). It would be more appropriate to study the silica-alumina ratio in colloidal material for these soils, since Anderson (1), Botkin (4), Bennett (3), and others have pointed out that the soil colloidal material varies apparently with the content of exchangeable bases and with the ratio of  $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3}$ . This, however, will be made a separate study in the near

future, together with some other factors involved in the physicochemical state of a slick soil.

Hydrogen-ion determinations (table 3) show a distinct gradation for the soils used, with somewhat higher values for the slick soil. These differences, however, are not large enough to attach any significance to these data, since a pH in a soil as high as 8 is still considered a favorable medium for plant growth.

In a preceding paragraph two reasons were given for a poor production of plant material. In both cases the real cause can be traced back to the poor physical condition of the soil. Losses of water take place because moisture does not penetrate into the soil and on prolonged standing on the surface of the soil is evaporated. In a soil with a poor physical condition the root system of a plant cannot develop very effectively or utilize to the best advantage the nutrients available. Numerous investigators have shown that different cations are responsible for a certain physical condition of a soil. Anderson (1), Bayer (2), and Gedroiz (8, 9), have shown that Na-saturated colloidal material is more dispersed than that which is Ca-saturated. Mattson (20) showed that a Na clay swelled more than a Ca-saturated clay, and that the Na increased the charge of a colloid (18, 19). Anderson believes that each cation exerts a characteristic influence in proportion to the quantities present. Bayer, however, states that "the properties of the system are not directly proportional to the degree of substitution of any one base for another. This is especially true in the comparison of the effects of the monovalent and divalent ions." Puri (23) showed that soils saturated with different cations, although having about the same clay content, have different deflocculation factors and dispersion coefficients.

Hissink (13, 15), Kelley and Brown (16), and Singh and Nijhawan (26) suggest the ratio of divalent to monovalent ions as particularly important indicators of the properties of the soils. Burgess and Breazeale (6) state, "little or no idea of the degree of dispersion of a soil can be gained from the study of the Ca:Na ratio." Data obtained on our soils show a positive correlation between the Ca:Na ratio and the amount of water necessary to produce a gram of dry weight of crop. Whether the deficient plant growth is due to a direct toxic effect of a high replaceable sodium content, as suggested by Kelley and Brown (16, 17), or due to the unfavorable physical condition of the soil, is difficult to tell. Further work is necessary to clear up this point. Probably both factors have an effect, but it is very likely that the latter is more important.

This last point could be partly cleared up by making a mechanical analysis of a soil to see to what extent the fine material predominates over the coarser fraction in the slick soil as compared with the normal soil. The mechanical analyses of these soils are given in table 4. It was of particular interest to run these analyses because of the work done at this station by Peterson, in which he found no appreciable difference in the mechanical composition of slick and normal soils. According to our data there is an increase in the fraction com-

prising the particle size beginning with the 1.00–0.5 mm. group and down through the 0.1–0.05 mm. groups in the normal soil over that of the slick soil. The fraction for the 0.05–0.005 mm. size remains constant at about 50 per cent, disregarding rather small discrepancies in a few samples. For the two fractions of 0.005–0.002 mm. size and the so-called colloidal fraction for particles smaller than 0.002 mm., the reverse is true, i.e. the larger proportion is found for the slick soil and the per cent of material decreases as the amount of normal soil increases over the slick. The colloidal fraction for the slick soil is about three times that of the normal soil. This varies inversely with the Ca:Na ratio in the base-exchange complex.

TABLE 6  
*Dispersion of clayic acid and various clayates\**

SOIL	ACIDOID OR SALOID	CLAY CONTENT	D. F.	D. C.
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Hoos fallow 18–27" ....	Clayic acid	33.9	1.0	2.9
	Na clayate	35.56	34.9	98.1
Rothamsted.....	K clayate	30.8	3.1	10.1
	Ca clayate	31.1	3.6	11.6
	Ba clayate	32.1	3.7	11.5
	Al clayate	32.3	1.7	5.3
Dhrwar soil.....	Clayic acid	60.5	1.4	2.3
	Mg clayate	60.5	3.2	5.3
	Ca clayate	60.5	2.2	3.6
	Ba clayate	60.5	2.6	4.3
	Al clayate	60.5	0.5	0.8
Ahola soil.....	Clayic acid	60.6	2.5	4.1
	Ca clayate	60.6	3.0	4.9
	Ba clayate	60.6	3.2	5.3

\* Table 1 from Puri (23).

Puri (23) in his studies of factors influencing the dispersion of soil colloids in water derives two factors—the dispersion factor and the dispersion coefficient. He states, “the degree of dispersion was measured by determining the percentage of conventional clay (0.002 mm. diameter) by the pipette method after leaving the soil in contact with water for 24 hours (referred to as dispersion factor or D. F.) and expressing it as percentage on the total clay content of the soil obtainable on complete dispersion. This is referred to as dispersion coefficient (D. C.) or  $D. C. = \frac{D. F. \times 100}{\text{Clay Content}}$ ”

The dispersion factor was determined in the samples used in this study (table 5). The results obtained from this study were closely correlated with the ratio of exchangeable Ca:Na in the soils. However, when the D. C. was

determined, using the colloidal clay for the soil from table 4, somewhat erroneous results were obtained. To illustrate the point, table 1 from Puri's paper (23) is given as table 6.

In this table he finds that the Na Clay has a D. C. of almost 100 per cent, and that Ca and other clays have a very low D. C. Singh and Nijhawan (26) find somewhat the same relation. They make the statement, "it seems feasible to seek for a relationship between the dispersion coefficient of soils under examination and the Ca:Na ratio in the exchange complex." In our work a different relation was found.

The slick soil has a higher D. F. than a normal soil, but the decrease of clay for the normal soil over the slick soil in proportion to the D. F. for those two soils is much larger for the normal soil. For that reason the D. C. proves to be larger for the normal soil, whereas according to Puri's work it should be smaller.

It can be seen, then, that the D. C. might give an erroneous idea of the physical state of the soil. The D. F., on the other hand, expresses the condition of the soil very well, and gives a simple and rapid way of classifying a soil.

#### SUMMARY

A set of soils was made up to study the relation among plant growth, water consumed by the plants, ratio of exchangeable Ca:Na in the soils, and the mechanical composition of the soil.

Chemical analyses and pH determinations made on slick and normal soils show very little difference between those two soils; they are also low in water-soluble salts.

There is a marked difference in the Ca:Na ratio in the exchange complex of the soils studied. The ratio for slick soil is low, 1.71; for the normal soil, very high, 48.40.

The mechanical analysis of the soils made shows that the colloidal fraction of a slick soil is about three times as great as that of a normal soil.

The amount of water necessary to produce 1 gm. of dry matter for the slick soil is about three times that for the normal soil.

Low crop producing power of the slick soil is attributed to the poor physical condition of this soil, which in turn is influenced by a relatively high exchangeable Na content.

The deflocculation factor of Puri expresses the physical condition of a soil; the dispersion coefficient runs contrary to what was expected.

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# INFLUENCE OF EIGHT YEARS' SUCCESSIVE FERTILIZING WITH CONCENTRATED POTASH SALTS AND WITH KAINIT ON THE REPLACEABLE BASES OF THE "TERRA ROXA" SOIL

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The study of the replaceable cations of the soil has been the subject of important papers by Gedroiz (3), Hissink (8), Ramann (21), Kappen (13), Kelley (15), Wiegner (23), de Sigmond (22), Page (18), Gehring (6), and many others. These studies point out very clearly the influence of the replaceable base content of the soil on its physicochemical and biological properties and therefore also on its fertility.

In this paper are published the results obtained in several experiments designed to determine the influence of potassium chloride, potassium sulfate, kainit, sodium chloride mixed with magnesium sulfate, and magnesium sulfate alone on the replaceable bases of the "Terra Roxa"<sup>1</sup> soil of our experimental plots (Santa Eliza Farm) which have been fertilized in the same way for 8 successive years.

## PRELIMINARY INFORMATION

The experimental plots for the study of the effect of potash fertilizers at Santa Eliza Farm were established November, 1925, on an exhausted soil very poor in nutritional elements but which, because of its good physical properties and its favorable topography, suited our purpose very well.

The chemical composition of this "Terra Roxa" is as follows:

ORGANIC MATTER	H <sub>2</sub> O AT 110°C	SiO <sub>2</sub>	TiO <sub>2</sub>	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>	MnO	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	SO <sub>3</sub>	Cl
per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent		per cent
10.94	3.25	37.52	5.37	21.95	19.56	0.11	0.29	0.05	0.11	0.94	0.11	Trace	0.02

Its physical constitution is as follows:

SAND 1-0.2 MM.	FINE SAND 0.2-0.04 MM.	SILT 0.04-0.01 MM.	FINE SILT 0.01-0.002 MM.	CLAY + SUBSTANCES BELOW 0.002 MM.	HUMUS	MOISTURE CONTENT
per cent	per cent	per cent	per cent	per cent	per cent	per cent
16.0	13.05	0.40	15.64	30.07	2.12	15.71

<sup>1</sup> Red soil resulting from the decomposition of augite-porphyratic rocks (1).

The experimental ground was divided into a series of plots 5 by 10 m., each plot being repeated six times in the field.

The fertilizers applied to the plots of the different series are shown in table 1.

The nitrogen and phosphoric fertilizers were always applied in the form of nitrate of soda and superphosphate (18 per cent) respectively. Since 1925

TABLE 1  
*Fertilizer treatment of plots*  
Each plot measured 50 sq. m.

SERIES NUMBER	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	N	CaO	MgO	Na <sub>2</sub> O	REMARKS
	gm.	gm.	gm.	gm.	gm.	gm.	
1	...	...	...	...	...	...	
2	...	450	400	7,980	...	...	The calcareous fertilizers were applied October, 1927, and in 1929
3	350	450	400	7,980	...	...	The K <sub>2</sub> O was applied in form of KCl
4	350	450	400	7,980	...	...	The K <sub>2</sub> O was applied in form of K <sub>2</sub> SO <sub>4</sub>
5	350	450	400	7,980	143	518	The K <sub>2</sub> O was applied in form of kainit
6	350	450	400	7,980	139	...	The K <sub>2</sub> O was applied in form of potassium and magnesium sulfate
7	...	450	400	7,980	143	518	The MgO was applied in form of MgSO <sub>4</sub> + 7H <sub>2</sub> O and the Na <sub>2</sub> O in form of NaCl
8	...	450	400	7,980	143	...	The MgO was applied in form of MgSO <sub>4</sub> + 7H <sub>2</sub> O

TABLE 2  
*Averages of 40 years' meteorological records for São Paulo*

Average temperature.....	19.7°C.
Vapor pressure.....	17.0 mm. Hg
Relative humidity.....	75.2
Relative deficit of saturation (100-75.2).....	24.8 per cent
0.248 x 17.04 mm. Hg.....	4.22 mm. Hg
Rainfall.....	1,409.1 mm.
Quotient $\frac{N}{S}$ .....	333.8
Average of maximum temperatures.....	26.2°C.
Average of minimum temperatures.....	14.5°C.
Absolute maximum temperature (14-10-901).....	36.7°C.
Absolute minimum temperature (14-7-92 and 19-8-902).....	0.2°C.

the following crops have been cultivated on these experimental grounds: corn, barley, corn, barley, cotton, corn, wheat, cotton, wheat, and rice. Before sowing each of these crop plants, we always applied the quantities of fertilizers indicated in table 1.

## CLIMATE

The climate of Campinas, which is more or less characteristic for the plateau of the State of São Paulo, can be distinctly divided into two different periods: one is dry and temperate (April to September) and the other is hot and moist (October to March).

The figures given in table 2, which represent average data of 40 years' observations at the Meteorological Observatory of this Agronomical Institute, indicate very clearly the characteristics of our climate.

The quotient  $\frac{N}{S}$  proposed by A. Meyer (17) gives an approximate idea of the humidity of a given region. A high coefficient indicates a humid climate, and a low one a dry climate. In order to obtain this quotient it is necessary to look up on a table of physical constants the vapor pressure which corresponds to the average annual temperature of the region. The figure thus obtained, expressed in millimeters of Hg, multiplied by the relative saturation deficit, gives us the absolute saturation deficit, which, divided by the quantity of rainfall during the year, expressed in millimeters, gives us the coefficient  $\frac{N}{S}$ .

## SOIL SAMPLING

The soil samples used in this study were taken at several places from the plots which make up a replication series, at a depth of 30 cm.; they were very well mixed together, passed through a 1-mm. mesh sieve, and then stored in hermetically closed glass bottles.

## EXPERIMENTAL METHODS

The pH values were determined in the aqueous suspension of the soil by the electrometrical method with a quinhydrone electrode; the exchange acidity was determined by the method of Daikuhara (2) and the hydrolitic by the method of Kappen.

The "buffer" capacity, which Kappen (12, p. 364) defines as the resistance of the soil to a change of reaction when one adds to it an acid or a base, was determined by the method of Jensen (11). In each of eleven 100-cc. Pyrex cylinders was placed 10 gm. of air-dried soil which had been passed through a 1-mm. mesh sieve. To cylinder No. 0 was added 10 cc. of boiled water, and to the others, 1 to 10 cc. of 0.1 *N* HCl or 0.1 *N* Ca(OH)<sub>2</sub> the volume being completed with distilled and boiled water. All the cylinders were plugged with rubber stoppers and left undisturbed for 24 hours. Afterwards the pH values were determined for each of them.

In order to present graphically the neutralization curve of each of the soils, we drew graphs locating on their abscissas the quantities of acid or of base added to each of the tubes expressed in cubic centimeters, and on the ordinates the pH values found in each case. For comparative purposes we drew for

each soil a second curve following the aforementioned method, taking instead of soil very fine pure sand, very well washed with acid.

The area located between these two curves, measured with a planimeter and expressed in square centimeters, represents the "buffer" surface of the soil.

"Buffer number" represents the distance measured on the abscissa between the bases of two perpendicular lines, the first of which passes through the point

TABLE 3

*Hydrogen-ion concentration of soil samples treated with different quantities of 0.1 N HCl*

NUMBER OF THE PLOTS	HYDROGEN-ION CONCENTRATION OF SOIL SAMPLES TREATED WITH										
	0 cc. 0.1N HCl	1 cc. 0.1N HCl	2 cc. 0.1N HCl	3 cc. 0.1N HCl	4 cc. 0.1N HCl	5 cc. 0.1N HCl	6 cc. 0.1N HCl	7 cc. 0.1N HCl	8 cc. 0.1N HCl	9 cc. 0.1N HCl	10 cc. 0.1N HCl
	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
7	6.72	6.52	5.72	5.36	4.81	4.45	4.11	3.91	3.54	3.36	3.09
8	6.57	6.23	5.46	5.03	4.61	4.32	3.93	3.60	3.42	3.19	3.04
10	6.52	6.03	5.22	4.54	3.85	3.47	3.16	3.02	2.98	2.82	2.61
11	6.56	6.33	5.37	4.86	4.53	4.10		3.49	3.21	3.12	2.89
12	6.60	6.08	5.20	4.73	4.22	3.80	3.47	3.23	3.11	3.02	2.79
13	6.45	6.39	6.01	5.44	5.12	4.76	4.46	4.36	3.95	3.75	3.48
14	5.82	4.96	4.08	3.65	3.23	3.00	2.83	2.80	2.64	2.50	2.41

TABLE 4

*Hydrogen-ion concentration of soil samples treated with different quantities of 0.1 N Ca(OH)<sub>2</sub>*

NUMBER OF THE PLOTS	HYDROGEN-ION CONCENTRATION OF SOIL SAMPLES TREATED WITH										
	0 cc. 0.1N Ca (OH) <sub>2</sub>	1 cc. 0.1N Ca (OH) <sub>2</sub>	2 cc. 0.1N Ca (OH) <sub>2</sub>	3 cc. 0.1N Ca (OH) <sub>2</sub>	4 cc. 0.1N Ca (OH) <sub>2</sub>	5 cc. 0.1N Ca (OH) <sub>2</sub>	6 cc. 0.1N Ca (OH) <sub>2</sub>	7 cc. 0.1N Ca (OH) <sub>2</sub>	8 cc. 0.1N Ca (OH) <sub>2</sub>	9 cc. 0.1N Ca (OH) <sub>2</sub>	10 cc. 0.1N Ca (OH) <sub>2</sub>
	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
7	6.72	7.14	7.31	7.48	7.62	7.87	7.97	8.10	8.22	8.82	9.07
8	6.57	7.81	7.20	7.40	7.60	7.80	8.16	8.22	8.45	8.50	
10	6.52	6.88	7.10	7.32	7.52	7.75	7.98	8.21	8.23	8.69	8.70
11	6.56	7.03	7.15	7.40	7.55	8.20	8.26	8.35	8.45	8.63	8.87
12	6.60	6.95	7.19	7.24	7.81	8.12	8.43	8.65	8.99	9.07	9.19
13	6.45	6.99	7.10	7.26	7.60	8.23	8.42	8.48	8.60	8.74	8.77
14	5.82	6.52	6.88	7.03	7.30	7.66	7.93	8.00	8.10	8.27	8.47

where the parallel to the abscissa, traced from the extremity of the soil curve, meets the basic curve, the other of which starts at the beginning of the soil curve.

Kappen (12, p. 317) thinks that the method of Jensen gives good results for determining the "buffer capacity" of the soil when one wants to find only the "buffer surface"; he believes, however, that the "buffer number" thus obtained, does not always show up the real buffer capacity and the other acidity values of the soil.

The determinations of the "buffer capacity" of the soil from the different plots, made as mentioned, revealed the pH values shown in tables 3 and 4, which were used to draw the neutralization curves and to calculate from them the "buffer surface" of each of them.

The total bases were determined by the methods of Gedroiz (4) and Hissink (9), and the saturation capacity of the soil by the methods of Hissink (10, p. 137) and Gehring, Pegau, and Wehrmann (7). We determined the quantity of Ca adsorbed by the soil, as Hissink proposes, as follows: 25 gm. of fine soil was placed in a 200-cc. beaker to which was added 100 cc. of a normal solution of NaCl. It was left to digest for 24 hours in an oven kept at 60°C. The following day the liquid of the beaker was thrown on a filter to be drained into a 1-liter graduated flask, then all the soil was quantitatively transferred to the filter. The residue was then leached with successive portions of a normal solution of NaCl (cold), until 1,000 cc. of filtrate was obtained to fill the flask. The filter was then removed to another liter flask, and the leaching with the normal solution of NaCl was continued until this second flask was filled.

TABLE 5

*Buffer surface and buffer number of soil samples from the experimental plots*

	NUMBER OF THE PLOTS						
	7	8	9	10	11	12	13
Buffer surface.....	11.1	10.0	4.6	7.2	8.9	8.2	11.7
Buffer number .....	8.8	8.7	6.4	7.2	8.6	8.4	9.0

In the first liter we find all the calcium adsorbed and a small portion in the form of carbonate; the difference between the quantities of calcium found in the first and second liter flask gives us the quantity adsorbed by the soil. In the present experiment the quantity of calcium found in the second liter flask was insignificant.

The determination of the maximum quantity of bases which a given soil is capable of adsorbing was made by the method of Gehring, Pegau, Wehrmann (5) as follows: 25 gm. of fine soil was added to 100 cc. of a saturated solution of calcium hydroxide and heated to 60°C. The thermometer used to measure the temperature was washed with 5 cc. of distilled water, which was added to the liquid in contact with the soil, the volume of which was thus increased to 105 cc. After the flask had been left undisturbed for 24 hours, to the liquid was added a few drops of phenolphthalein, which should tinge the liquid red. This did not happen, however, because the soil had adsorbed all the calcium from the 100 cc. of  $\text{Ca}(\text{OH})_2$  of the first addition. Twenty cubic centimeters more of the saturated solution of calcium was added, and the flask was again left undisturbed. Afterwards some  $\text{CO}_2$  gas was passed through the liquid until its color changed from red to colorless. Then the soil suspension was heated for some time, enough NaCl being added to keep a normal solution. The

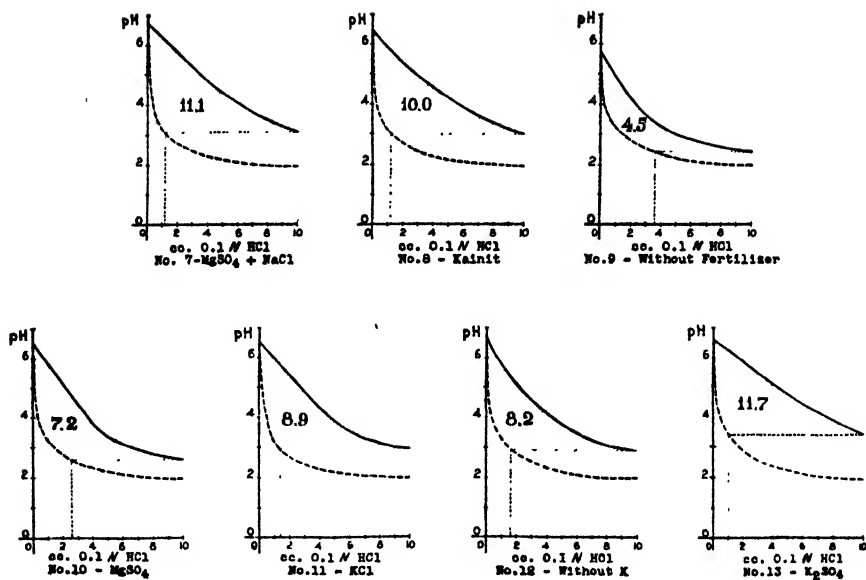


FIG. 1. GRAPHS OF BUFFER SURFACE AND BUFFER NUMBERS OF SOIL SAMPLES TREATED WITH DIFFERENT QUANTITIES OF TENTH-NORMAL HYDROCHLORIC ACID

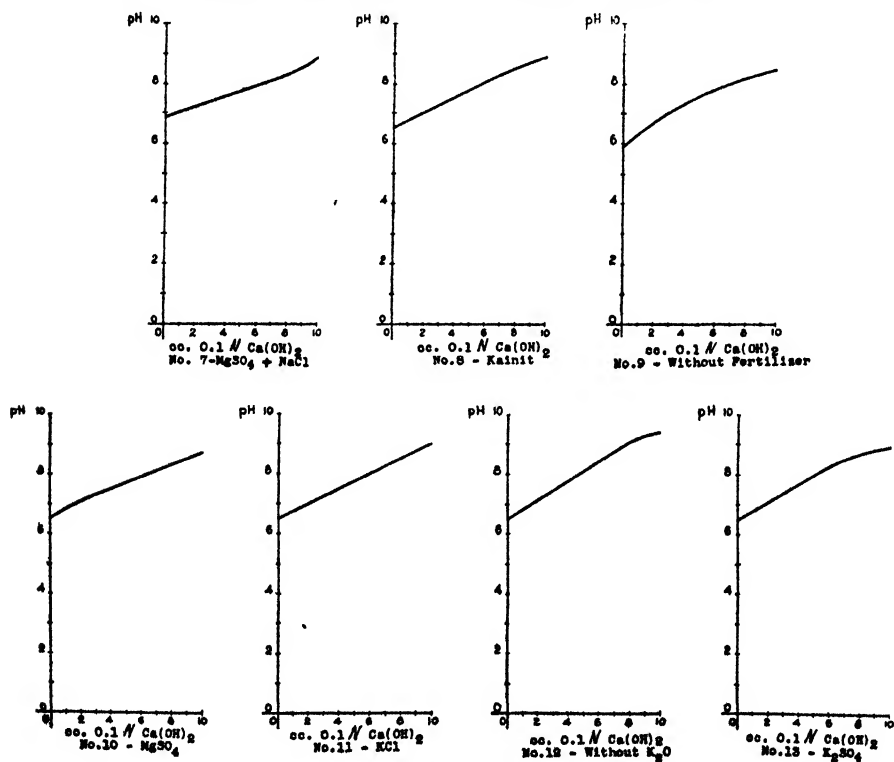


FIG. 2. THE pH VALUES OF SOIL SAMPLES TREATED WITH DIFFERENT QUANTITIES OF TENTH-NORMAL CALCIUM HYDROXIDE

TABLE 6  
Comparison of exchangeable bases in soil samples from the different plots

	NUMBER OF THE PLOTS	Al <sub>2</sub> O <sub>3</sub>		CaO		MgO		MnO		Na <sub>2</sub> O		K <sub>2</sub> O	
		mgm.	m.e.	mgm.	m.e.	mgm.	m.e.	mgm.	m.e.	mgm.	m.e.	mgm.	m.e.
NaCl + MgSO <sub>4</sub> .....	7	0.0954	5.618	0.3123	11.140	0.0254	1.253	0.0237	0.622	0.0364	1.174	0.0107	0.227
Kainit.....	8	0.1006	5.923	0.3038	10.835	0.0195	0.963	0.0315	0.828	0.0381	1.229	0.0176	0.373
MgSO <sub>4</sub> .....	10	0.0968	5.695	0.2833	10.106	0.0211	1.052	0.0249	0.654	0.0339	1.094	0.0097	0.205
KCl.....	11	0.0998	5.873	0.2904	10.358	0.0133	0.658	0.0310	0.801	0.0371	1.196	0.0195	0.412
Without K <sub>2</sub> O.....	12	0.099	5.927	0.2938	10.479	0.0137	0.681	0.0308	0.808	0.0335	1.081	0.0095	0.201
K <sub>2</sub> SO <sub>4</sub> .....	13	0.0965	5.680	0.3249	11.592	0.0158	0.785	0.0312	0.818	0.0339	1.094	0.0196	0.406

TABLE 7

Value for  $T$ ,  $S$ ,  $T-S$ ,  $100 \frac{(T-S)}{T}$ , and  $V = \frac{100 \cdot S}{T}$  as determined by the method of Hissink modified by Gehring, Pegau, Wehmann

	NUMBER OF THE PLOTS	HYDROLYC ACIDITY $\gamma_1$	ACIDITY DAIKUHARA $\gamma_1$	pH	CLAY + FINE SILT	MAXIMUM SATURATION CAPACITY $T$		REPLACED Ca S		SATURATION DEFICIT $T-S$		SATURATION DEFICIT $\frac{(T-S)}{T}$	$\frac{S}{\text{CLAY} + \text{FINE SILT}}$	DEGREE OF SATURATION $V = 100 \times \frac{S}{T}$
						CaO	m.e.	CaO	m.e.	CaO	m.e.			per cent
NaCl + MgSO <sub>4</sub> .....	7	....	....	6.72	47.11	0.4485	16.020	2702	9.650	0.1783	6.37	39.81	0.20	60.2
Kainit.....	8	11.83	0.14	6.57	48.70	0.3869	13.820	2668	9.530	0.1201	4.29	31.03	0.19	69.0
MgSO <sub>4</sub> .....	10	....	....	6.52	49.70	0.5384	19.230	2742	9.790	0.2642	9.43	48.99	0.19	50.9
KCl.....	11	13.31	0.13	6.56	46.61	0.3802	13.580	2789	9.960	0.1013	3.62	26.65	0.21	73.3
Without K <sub>2</sub> O.....	12	11.55	0.20	6.60	46.21	0.5630	20.110	2707	9.670	0.2923	10.44	50.91	0.20	48.0
K <sub>2</sub> SO <sub>4</sub> .....	13	12.14	0.15	6.45	49.18	0.5807	20.740	2833	10.120	0.2974	10.62	51.20	0.20	48.8



liquid was then decanted and the soil was placed quantitatively on a filter above a liter flask and leached with a normal solution of NaCl (cold) until 1,000 cc. of the filtrate was obtained. Afterwards the filter with the soil was transferred to a second liter flask, and the leaching continued until the flask was filled. The quantities of calcium in the first and in the second liter flasks were determined. The difference between these quantities is the maximum quantity of calcium which the soil can adsorb (tables 6 and 7).

In order to calculate the degree of saturation of the soil, Hissink (10, p. 143) uses the following formula  $V = \frac{100 S}{T}$ , in which  $V$  represents the saturation capacity of the soil in percentage,  $T$  the maximum quantity of a base which a soil can adsorb, and  $S$  the quantity of adsorbed calcium which the soil contains.

#### DISCUSSION

The continued use of potassic fertilizers, intensifying the concentration of K cations in the soil solution, modifies the relation among the adsorbed bases. This occurs because the potassium replaces these bases from the humic and zeolitic compounds of the soil, making them much more readily exchangeable, as seen in table 8.

As specified in the table, the dominant base is calcium, with the maximum percentage of 84.2 per cent for the series, without potash. Sodium takes second place with the maximum percentage of 9.4 per cent in the potassium chloride series.

It is noteworthy that the application of sodium chloride did not increase the percentage of adsorbed sodium, as might have been expected, for the soil samples which did not receive sodium chloride contained as much adsorbed sodium as the others which did get an addition of this salt. The anion of the salt proved to have a great influence, as the potassium sulfate series contains 2.3 per cent less sodium than the chloride series.

The series which received magnesium, on the contrary, showed greater content of adsorbed magnesium than the series which did not receive it. Nearly all the series without magnesium show almost the same proportion of this salt, that is, 5.2 per cent. It is also very interesting to follow the way in which the potassium acts in the soil. All series which received potassic fertilizers contained greater proportions of this cation in the adsorbed state than did those without potassium. But the series in which the salt was applied in the form of chloride contained a greater proportion of potassium than did the sulfate series.

#### *Adsorbed bases and state of saturation*

A comparison of the chemical analyses of the soil samples from the plots of the chloride, potassium, sulfate, and kainit series shows that the percentage of adsorbed calcium varies as follows: 0.293 per cent in the series without potassium; 0.303 per cent in the series with kainit; 0.290 per cent in the series with chloride; 0.325 per cent in the series with potassium sulfate.

The influence of the anion of the salt is very clear in this case, that is, the series which received chloride contained less adsorbed calcium than the ones which received sulfate. On the contrary, Page and Williams (19) found no considerable differences in the percentage of replaceable calcium among the various plots of Broadbalk which have been annually fertilized now for 82 years, probably because the soil is well supplied with calcium.

But small differences were noted between the influences of kainit and chloride; the amount of adsorbed calcium found in the soil of the plots which received such potassic fertilizers is approximately the same as that found in soil samples of plots without potassic fertilizer applications.

Similar observations were made concerning magnesium.

Noteworthy, also, is the following observation: Studying the changes of the capacity of the soil to adsorb bases, this experiment showed that this capacity of the soil in different series increased very considerably in 2 years. In 1929 the plots of the series of chloride, kainit, and without potassium showed re-

TABLE 8

*Relative proportions of different bases in plots receiving several kinds of fertilizers*

NUMBER OF THE PLOTS	TOTAL BASES	Ca	Mg	Na	K
	<i>mgm</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
7	13.79	80.7	9.08	8.5	1.6
8	13.40	80.8	7.20	9.1	2.7
10	12.45	81.2	8.40	8.7	1.6
11	12.60	82.07	5.20	9.4	3.23
12	12.44	84.2	5.30	8.6	1.60
13	13.88	83.5	5.60	7.1	2.90

spectively the following percentages of total bases: 6.49, 5.64, 6.24, and 6.66 m.e., as determined by the Bobko and Askinasi method. After the experiment these plots contained respectively 12.6, 13.88, 13.4, and 12.44 m.e., as determined by the Gedroiz method. Thus the increases are: for the potassium chloride series 6.11 m.e., for the sulfate series 8.24, for the kainit 7.16, and for the series without potassium 5.78 m.e.

The soils from the potassium chloride series contain less adsorbed magnesium than do those from the sulfate series. The soil samples from the chloride series are richer in sodium than are those of the sulfate series. Both series have, however, the same percentage of adsorbed potassium, as shown in table 8.

It is interesting to compare the percentages of adsorbed calcium, magnesium, sodium, and potassium existing in the soils of the sodium chloride plus magnesium sulfate series and of the kainit series with the soil sample from the series which received only magnesium sulfate. We noted approximately the same percentage of calcium in all the series. This did not occur with the replaceable magnesium and sodium, which were found in greater proportion in plots which

received an application of these salts. Neither the addition of magnesium nor of sodium had any influence on the percentage of adsorbed potassium, which remained approximately the same in the soil of all plots which did not receive potassic fertilizer.

Potassic fertilizer, as we have seen, showed a certain influence on the adsorbed bases, modifying also the capacity of the soil to adsorb bases and consequently also its state of saturation. It is interesting to note that the most saturated soils are those in the series receiving potassium chloride, 73.3 per cent, and the least saturated are those in the series without potassium, 48 per cent (table 7). The anion  $\text{SO}_4$  apparently contributes toward the lowering of the saturation state of the soil.

#### *Influence of potassium fertilizers on soil acidity*

The potassium salts employed as fertilizers are physiologically acid, according to A. Mayer (16), because the cations are generally absorbed by the roots of the plants, the anions remaining in the soil. The continued use of these salts, therefore, on the same soil should render it acid after a certain period of time. This, however, does not happen, as several experiments have shown. Kappen (14) explains this fact by assuming that the plants adsorb a great deal of the anions from the employed fertilizers. He found in his experiments that plants absorbed about 50 per cent of Cl from the chlorides added to the soil. Prianischnikow (20) concluded that the plants differ markedly in this respect; he found, for instance, that oats and barley absorb cations and anions at approximately the same rate, whereas the maize plant absorbs nearly three times as many cations as anions.

Prianischnikow's conclusions are thus not in agreement with those of Kappen's. Furthermore, it must be kept in mind that the phenomenon of absorption in the soil is quite different from that in a nutrient solution. In the soil the bases are replaced in equivalent quantities, following the mass law, and the adsorption of one is always accompanied by the replacement of another or by hydrogen which is found in the absorption complex of unsaturated soils.

In our experiments the series of plots which received no potassium fertilizer but which was supplied with the same quantities of superphosphate, sodium nitrate, and calcium, has approximately the same pH index as the plots which received an application of potassium chloride and kainit. But this series which received no potassium is much less acid than the soil of the series which received potassium sulfate, as we see in table 7; the series without potassium has a pH index of 6.6, whereas the soil samples of the sulfate series have a pH index of 6.45, which shows them to be more acid. The hydrolytic acidity is a bit stronger in the series which received the potassium chloride and sulfate, but the Daikuhara acidity is weaker in both series.

## SUMMARY

In this paper is discussed the influence of 8 years' successive fertilizing with concentrated potash salts and with kainit on the replaceable bases of the "Terra Roxa" soil.

A considerable increase in the soil's capacity to adsorb bases was revealed in the soils of all the experimental plots; this increase was most significant in the soils of the potassium sulfate series, followed by lower increases respectively in the series of kainit, potassium chloride, and without potassium.

It was also shown that the soil from the plots which received potassium chloride and kainit contained a higher proportion of adsorbed potassium than the soil from the sulfate series.

The soil in the potassium chloride series is more saturated with bases (73.3 per cent), whereas that in the potassium sulfate series contains only 48.8 per cent of bases.

We may conclude, therefore, that the anion of the salt plays a very important rôle in the effect of potash fertilizers in our "Terra Roxa" soil. The sulfates contribute more than the chlorides to decrease the state of saturation of the soil.

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# THE RELATION OF COLLOID DISPERSION IN SOILS TO CHEMICAL CHANGES INDUCED BY BIOLOGICAL TRANSFORMATIONS OF ORGANIC MATERIALS<sup>1</sup>

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The effect of organic matter on the physical properties of soils has been abundantly demonstrated by practical experience and scientific experiment. Many of its influences, especially those of manure, were known to the ancients (16). Schübler has been credited by Keen (26) with being the first to show by laboratory experiments the effects of the presence of this material in the soil on its physical properties.

Warington (43), Russell (36), and Keen (26) discuss the beneficial effects of added organic matter on the physical properties of the soil. Keen attributes its favorable action to the improvement in soil tilth, rather than to a reduction in soil resistance, as measured by the draft of agricultural implements. Russell also attributes to humus a marked improvement in the tilth and general mechanical condition as well as an increase in moisture-holding capacity.

Many more citations, which would vary but slightly from the foregoing, could be given as to the beneficial effect of organic matter on the physical condition of the soil. There are, however, a few which can be interpreted to show that certain types of organic matter may have a much less beneficial, or even an injurious, effect. Sievers and Holtz (38) observed "the beneficial effects on the physical condition of the soil—generally resulting from the incorporation of organic matter in direct proportion to its nitrogen content." Legumes were found to be much more effective than non-legumes.

Chapman (13) reported that after seedbed preparation, only 10.8 per cent of the surface soil of the timothy (rotation: corn, barley, timothy, wheat) plot passed through a 1/16-inch mesh screen, whereas 55.7 per cent from the clover (rotation: corn, barley, clover, wheat) plot passed through it. The soil crumbs from the timothy plot were sharp-edged whereas, those from the other were rounded, irregular, and far from granular. To account for his results, he suggested three possible causes; namely, granulation on wetting and drying set up by the different stresses of organic and mineral soil material; weaknesses developed by the decay of the organic matter, producing lines of cleavage; and qualitative and quantitative differences in the concentration of ions in the soil brought about by the difference in plants grown. The last he considers the least likely.

Stephenson (39) in studying the effect of different organic materials on soil reaction found that albumin and casein added to soils and incubated made them hard, lumpy, and difficult

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to handle even in the presence of lime. Suspensions from them passed through two thicknesses of filter paper. Additions of blood caused similar results in the early part of the experiment, but later the filtrates were clear. The addition of carbohydrate materials, especially starch, caused the soils to become sticky and "of a consistency similar to putty." The alfalfa-treated soils remained "granular and in good tilth."

Thus, it appears that not all types of organic matter have the same effect on the physical properties of a soil. Inasmuch as the chemical properties of the materials which cause such divergent physical conditions in the soil are so markedly different, it would seem that these substances can show their influence in at least two ways.

On the one hand, organic material operates directly or statically by its mere presence in the soil. The properties which it imparts in this regard are largely additive, i.e., they may be considered as a summation of the physical and colloidal properties of the organic substances and of the mineral soil particles, with due regard, of course, for size and aggregation of units. Its influence in this respect is felt as long as the organic material itself remains. Bayer (4) removed a considerable part of the organic matter from soils by oxidation with peroxide according to Robinson's (35) method, thereby obtaining lower absorptive capacities for cations and for hygroscopic moisture as well as lower moisture percentages for the upper and lower plastic limits.

On the other hand, organic matter may influence the soil indirectly or dynamically by the changes which it induces. The properties which it imparts to the soil in this way have very little relation or none whatever to its own physical properties. Its effects are not additive to those of the mineral soil colloids but result from the microbiological activity caused by its presence in the soil. The compounds making up the organic matter, although they are themselves largely non-electrolytes, influence the physical properties by bringing about changes in the electrolyte concentration of the soil solution.

Organic materials resistant to decay largely exert their influence statically; those which quickly decay show their effects dynamically.

Much attention has been given to the rôle that electrolytes play in coagulating, flocculating, and dispersing soil colloids in clay suspensions. Recently Keen (26) has summarized them. Nitrification of organic materials and fallowing have increased electrolyte concentrations in soils (3, 10, 11, 12, 27, 41). Higher plants and microorganisms furnished with carbonaceous materials have reduced the electrolyte concentrations (10, 11, 12, 15, 23, 41). Sufficient change in the concentration of the soil solution, biologically produced, may alter the stability of the soil suspension. This has been sufficient, with higher plants at least, to produce changes in the dispersion of the soil colloids (2, 3, 17, 22, 31). With respect to the dynamic rôle of organic materials in soils, however, there is need for a study of the quantitative interrelationships of the rate of application of material, its chemical composition, duration of incubation, concentration of various ions, and the accompanying physical properties as applied to given soils.

#### PLAN OF THE EXPERIMENTS

The soils, a Fresno fine sandy loam from a vineyard in Fresno County and a Yolo loam from a fallowed field in the experimental area at Davis, were carefully prepared for laboratory work by drying, crushing of the larger clods, sifting, and thoroughly mixing. Of the varied amounts of urea added, the greatest was sufficient when fully nitrified to precipitate practically all of the colloids in suspension as measured by the procedure described in the following.

Samples of each of these soils were treated with 50 m.e. of  $\text{HNO}_3$  per kgm. of dry soil in the case of the Fresno soil, and 25 m.e. in the case of the Yolo soil. Samples thus treated are referred to as "nitrated" Fresno fine sandy loam and "nitrated" Yolo loam respectively. After drying, each soil was again sifted

and thoroughly mixed. Sucrose was added to these samples in increasing amounts, the largest addition being sufficient to disperse the soil colloids materially.

The treatments for the various samples of soil were as follows:

SOILS	TREATMENTS					
	<i>Ucea—m.a. of nitrogen</i>					
Fresno fine sandy loam.....	0	10	20	35	60	
Yolo loam.....	0	4	8	15	30	
	<i>Sucrose—m.a. of carbon</i>					
Fresno fine sandy loam "nitrated".	0	75	150	300	750	
Yolo loam "nitrated".....	0	50	100	200	400	750

\* Milligram-atoms per kilo of soil.

Samples were incubated in a room thermostatically controlled at about 28°C.

The analyses reported in the various tables were made by methods generally used in such determinations, all being volumetric, gravimetric, or electrometric. The determinations of  $\text{NH}_4$  and  $\text{NO}_3$  ions in the *N* KCl extract at pH 1 were made as described by Russell<sup>2</sup> (36, p. 567). Silica was determined as usual except that the final volatilization with HF was omitted.

#### *Methods for suspended and soluble solids*

In the preliminary trials, many criteria for the physical properties of the soils were examined. None of them was so easy of manipulation or permitted such good duplication of results as that of suspended solids. Although Puri and Keen (34) noticed a reduction in the amount of suspended material when the soil was increased much over 2 per cent of the water, it seemed desirable on the basis of preliminary tests, to take a concentration of 10 per cent.

Twenty-five grams of dry soil (or its equivalent in moist soil) was churned with 250 ml. (less the water in the soil) of distilled water, previously kept at a temperature of 30°C. This mixing was accomplished in an Arnold #16 sanitary drink mixer, in which the propeller had been directly attached to the motor shaft, and the lower bearing of which had been removed. Part of the suspension was poured quickly into a long glass tube (23 mm. in diameter) and placed in a 30°C. constant temperature chamber for 24 hours. The remainder of the suspension was then thrown on a filter to obtain the extract used for the determination of soluble solids and specific conductance. Total solids in each case were determined by withdrawing 20 ml. of suspension with a pipette, the tip of which was held 10 cm. below the original surface of the liquid, evaporating and drying. Suspended solids equal total solids minus soluble solids.

<sup>2</sup> In the nitrate determinations after the first distillation more water was added and distillation continued to secure all of the nitrates.



The churning period was 5 minutes in each case. Longer churning increased the dispersion, as shown by Puri and Keen (34), Bradfield (6), and Bouyoucos (5), as well as by our own preliminary results. This length of time was arbitrarily chosen because it gave quite reproducible results, and was not too time-consuming.

### *Hydrogen-ion concentration*

These determinations were made with the quinhydrone electrode in a bath at 25°C., on 1:1 soil suspensions with H<sub>2</sub>O and with *N* KCl. In general, the tests with the aqueous suspensions were made in quadruplicate and those with the *N* KCl in triplicate. The results are expressed in terms of pH. Ten second values were used in making readings.

### UREA TREATMENTS

The effects of the decomposition of urea on Fresno fine sandy loam as determined for the various incubation periods are given in table 1. No conspicuous differences in the dispersion are disclosed except where the largest amount of urea was added. Although the electrolyte content was considerably augmented as a result of incubation, it did not cause any clear-cut flocculation of the suspended solids, presumably because of the dispersing effect of the increasing concentration of OH ions and of the greater amount of NH<sub>4</sub> ions in the exchange complex. The failure to accumulate NO<sub>3</sub> ions with the larger applications of urea was clearly assignable to the inhibiting effect of the larger amounts of ammonia upon the nitrification processes.

In the amounts of suspended solids as given in the various tables in this paper, minor variations appear which cannot be correlated with changes in the concentrations of the various ions so far studied. These variations must be attributed to a factor, or factors, as yet undisclosed.

In the control soil and in that receiving 10 m.a. of nitrogen as urea, ammonification and nitrification went on normally. Initially the ammonia content increased, sending the pH up to 8.19 in the latter treatment. Subsequently, with the change of ammonia to nitrate, the pH was lowered below that of the control soil. With the change of urea to ammonia, the specific conductance increased to some extent, but the increase was still more marked with the transformation of ammonia to nitrates. With the larger amounts of urea, it is not so easy to tell what changes had been taking place in all respects. As the pH values were high for some time during the incubation period some of the nitrogen may have been lost from the containers by volatilization as ammonia.

More complete analyses of the Fresno fine sandy loam with various amounts of urea added were made after 4 weeks of incubation. The results of these are reported in table 2. No new points are brought out with regard to the suspended solids. The specific conductance of the 1:10 extract increased with increasing amount of urea; that of the 1:1 extract also increased with the exception of the treatment with the largest amount.

The concentration of bicarbonates rose with increasing amounts of urea. The K ions increased from 0.82 m.e. per kgm. in the control treatment to 1.67, with a falling off to 1.28 in the highest urea treatment. Sodium ions and Mg

TABLE 1

*Effect of the biological destruction of urea on Fresno fine sandy loam as measured at various times during the incubation*

UREA MGM-ATOM NITROGEN PER KILO	TIME OF INCUBATION WEEKS	1:10 = SOIL:H <sub>2</sub> O RATIO			QUIN pH AT 25°C. 1:1 SUSPENSION		N KCl EXTRACT AT pH 1 M.E PER KILO	
		Mgm in 20 ml.		Sp. cond. Mhos × 10 <sup>-4</sup>	H <sub>2</sub> O	N KCl	NH <sub>4</sub>	NO <sub>3</sub>
		Susp'd solids	Soluble solids					
0	3/7	26.5	1.4	0.84	7.33	6.36	1.06	0.6*
	2	25.8	2.6	1.02	6.68	6.16	0.35	1.5
	7	21.0	1.2	1.23	6.24	5.72	0.22	3.96
10	3/7	24.1	2.0	1.46	8.19	7.47	10.10	0.8
	1	25.5	1.4	1.60	6.70	6.24	5.81	2.5
	2	29.1	3.2	1.78	5.98	5.70	4.62	6.1
	4	18.8	4.2	2.02	5.32	4.99	1.29	8.40
	7	19.3	3.2	2.12	5.06	4.91	0.51	10.40
	16	20.4	3.2	2.32	5.28	4.96	0.80	13.00
20	3/7	26.2	2.0	2.00	8.48	8.09	19.7	0.9
	1	27.5	1.5	2.16	7.75	7.41	12.92	2.4
	2	26.3	2.9	2.26	6.54	6.14	10.72	6.2
	4	21.7	2.4	2.12	6.40	6.00	8.10	5.5
	7	20.7	2.5	2.11	5.82	5.57	6.31	8.92
	16	26.1	2.5	2.06	6.25	5.73	7.20	6.70
35	3/7	25.4	2.7	2.89	8.63	8.35	30.23	0.6
	1	27.5	2.0	2.88	8.53	8.23	27.02	0.6
	2	31.2	3.4	3.00	7.64	7.34	25.55	6.2
	4	22.7	2.7	3.00	6.56	6.28	14.55	6.8
	7	22.7	2.6	2.76	6.33	6.07	10.20	12.12
	16	28.0	2.7	2.77	6.50	6.07	14.25	8.20
60	3/7	29.7	3.1	4.20	8.73	8.53	57.2	0.4
	2	33.3	4.7	3.84	8.67	8.38	52.5	0.3
	7	32.4	3.9	3.17	8.65	8.33	43.4	0.52

\* Values in the last column for NO<sub>3</sub> given to one decimal place may be slightly low, as in their determination no second distillation was run. On those expressed to two places, after the contents of the flask were boiled down, additional water was added, the distillate caught and titrated separately, and the value added to the first.

ions were affected similarly to K ions, whereas Ca ions were a little more variable and were decidedly decreased by the largest urea treatment. The marked reduction of Ca and Mg ions in the highest urea treatment is consistent with the formation and precipitation of CaCO<sub>3</sub> and MgCO<sub>3</sub> in the soil at the pH

values which occurred in the soil at that time. The increase in phosphate may be ascribed in part at least to the decline in the concentration of the Ca and Mg ions. The  $\text{PO}_4$  ions diminished slightly with increasing amounts of urea, except in the case of the largest urea treatment, where it was higher. Sulfate ions increased slightly.

The results of a similar study of the effects of urea decomposition on Yolo loam are given in table 3. Here nitrification, even in the presence of larger

TABLE 2

*Effect of the biological destruction of increasing amounts of urea upon Fresno fine sandy loam after an incubation period of 4 weeks at 28°C.*

UREA ADDED, MGM.-ATOMS OF NITROGEN PER KILO SOIL. ....	0	10	20	35	60
1:10—suspension:					
Susp. solids. mgm. in 20 ml. ....	21.2	21.0	21.2	21.4	26.0
Extract—soluble solids. ....	2.8	3.8	3.5	3.8	4.6
Sp. cond. Mhos $\times 10^{-4}$ . ....	1.22	1.79	2.18	2.91	3.69
Quin pH 1:1 suspensions $\text{H}_2\text{O}$ . ....	6.19	5.77	6.19	6.43	8.43
N KCl. ....	5.72	5.55	5.85	6.12	8.01
Extract: 1:1 = soil: $\text{H}_2\text{O}$					
Sp. Cond. Mhos $\times 10^{-4}$ . ....	5.58	11.10	15.12	20.83	20.47
Ions—m.e./kilo:					
$\text{NH}_4$ . ....	0.24	1.18	4.20	7.94	17.52
K. ....	0.82	1.18	1.44	1.67	1.28
Na. ....	0.69	0.75	0.80	0.95	0.80
Ca. ....	2.32	4.55	4.19	4.44	1.47
Mg. ....	1.12	2.28	2.24	2.32	0.97
Total cations. ....	5.19	9.94	12.87	17.32	22.04
$\text{HCO}_3$ . ....	0.52	0.70	1.06	1.92	13.90
Cl. ....	0.67	0.73	0.78	0.67	0.70*
$\text{NO}_3$ . ....	2.84	7.30	9.92	14.56	5.22
$\text{SO}_4$ . ....	0.68	0.67	0.63	0.62	0.95
$\text{PO}_4$ . ....	0.60	0.57	0.44	0.45	0.95
Total anions. ....	5.31	9.97	12.83	18.22	21.72

\* The solution was too dark colored for titration. This value was assumed.

applications of urea, was not sufficiently inhibited to prevent almost complete transformation of the ammonia to nitrates during a period of 11 weeks. Transformations took place during the first 4 weeks of incubation, sufficient in amount with the treatment of 8 m.a. of nitrogen as urea, to cause a material reduction of the colloids in suspension. As shown in the table, the higher applications of urea for this and longer periods of incubation reduced the suspended solids practically to zero.

During the course of the incubation, the specific conductance of the 1:10

extract increased with time. With the control soil this tendency may be attributed to the formation of nitrates from the decomposition of the soil's original organic matter. In the other cases this was a factor which, also, became less and less proportionally as the amount of urea was increased. In all treatments, the specific conductance increased over the control by the trans-

TABLE 3

*Effect of the biological destruction of urea on Yolo loam as measured at various times during the incubation*

UREA MGM.-ATOM NITROGEN PER KILO	TIME OF INCUBATION WEEKS	1. 10 = SOIL:H <sub>2</sub> O RATIO			QUIN pH AT 25°C. 1:1 SUSPENSION		N KCl EXTRACT AT pH 1	
		Mgm. in 20 ml.		M.E. PER KILO				
		Susp'd solids	Soluble solids		Sp cond. Mhos × 10 <sup>-4</sup>	H <sub>2</sub> O		N KCl
0	1	103.2	1.0	1.02	7.46	6.62	0.31	1.2*
	2	93.6	1.2	1.17	7.16	5.77	0.26	2.46
	7	105.2	1.6	1.26	7.18	5.96	0.12	2.60
	11	100.6	1.8	1.19	7.14	6.01	0.24	3.70
4	1	93.5	1.8	1.27	7.34	6.46	1.98	2.7
	2	106.5	1.8	1.51	6.80	5.73	0.56	3.78
	4	94.0	2.5	1.58	6.87	5.89	0.36	6.30
	7	91.8	2.0	1.56	6.87	5.89	0.12	6.12
	11	87.1	2.5	1.58	6.87	5.90	0.48	6.60
8	1	102.1	2.0	1.40	7.32	6.53	5.05	3.1
	2	104.6	2.0	1.65	6.67	5.77	2.28	7.16
	4	17.7	3.4	1.94	6.63	5.80	0.21	9.49
	7	20.0	2.8	1.95	6.60	5.73	0.26	9.26
	11	12.3	3.2	1.96	6.60	5.72	0.36	8.90
15	1	98.0	1.7	1.60	7.57	6.82	11.22	2.7
	2	104.8	2.2	1.90	6.89	5.95	6.90	7.94
	4	2.8	4.6	2.62	6.33	5.66	1.22	15.10
	7	2.5	3.6	2.69	6.24	5.42	0.18	15.50
	11	0.4	5.3	2.76	6.36	5.47	0.30	16.54
30	1	87.8	1.8	2.10	8.26	7.34	22.60	2.3
	2	112.1	2.2	2.28	7.47	6.63	18.88	7.48
	7	-0.2	5.6	3.82	5.77	5.13	2.98	24.80
	11	-0.7	7.6	4.46	5.65	5.06	0.66	28.18

\* See footnote to table 1.

formations of the urea to ammonia, and still further increased by the change of ammonia to nitrates. The pH increased with the transformations of urea to ammonia, and then progressively decreased below the initial pH with the progressive change to nitrates.

The results of more complete analyses of the Yolo loam at the end of 11 weeks of incubation are given in table 4. Here, even in the highest urea treatment,

practically all of the  $\text{NH}_3$  was transformed to nitrates. At 11 weeks, the nitrification of 15 m.a. of N (and higher rates of application) as urea caused practically complete flocculation of the soil colloids in the 1:10 suspension. The pH declined, changing from the control with 7.13 to 5.70 in the largest urea application. In the 1:1 extract the specific conductance likewise increased, with progressively larger amounts of urea added, from 5.50 to  $30.5 \times 10^{-4}$  mhos. Except for  $\text{NH}_4$  all of the cations progressively increased with larger

TABLE 4

*Effect of the biological destruction of increasing amounts of urea upon Yolo loam after an incubation period of 11 weeks*

UREA: MG./ATOMS OF NITROGEN PER KG. SOIL .....	0	4	8	15	30
1:10—suspension:					
Susp'd. solids: mgm. in 20 ml.....	89.0	73.8	13.2	0.7	-1.3
Extract—soluble solids, do.....	1.5	2.3	3.0	4.7	7.6
Sp. cond. Mhos $\times 10^{-4}$ .....	1.24	1.72	2.04	2.80	4.41
Quin pH 1:1 suspensions $\text{H}_2\text{O}$ .....	7.13	6.85	6.62	6.29	5.70
N KCl.....	5.94	5.82	5.70	5.52	5.16
Extract: 1:1 = soil: $\text{H}_2\text{O}$					
Sp. cond. Mhos $\times 10^{-4}$ .....	5.50	9.24	13.10	19.45	30.5
Ions m.e./kgm.:					
$\text{NH}_4$ .....	0.06	0.16	0.14	0.14	0.14
K.....	0.24	0.37	0.39	0.54	0.70
Na.....	0.94	1.04	1.13	1.24	1.39
Ca.....	1.61	3.04	4.09	6.82	11.32
Mg.....	2.49	4.66	6.50	10.76	17.24
Total cations.....	5.34	9.27	12.25	19.50	30.79
$\text{HCO}_3$ .....	0.48	0.32	0.32	0.24	0.16
Cl.....	0.20	0.20	0.22	0.17	0.20
$\text{NO}_3$ .....	3.58	7.58	10.64	17.20	29.36
$\text{SO}_4$ .....	0.60	0.57	0.50	0.54	0.54
$\text{PO}_4$ .....	0.05	0.04	0.03	0.05	0.04
Total anions.....	4.91	8.71	11.71	18.20	30.30

applications of urea. With this particular soil in each 1:1 extract the concentration of Mg ions was higher than that of any other cation.

It is of interest to note that in these tests the rates of increase of equivalents of Ca and Mg ions was greater than those of the other basic cations, and that the rates of these two were practically equal. The rate of increase of equivalents of  $\text{NO}_3$  ions was slightly greater than those of Ca and Mg, although they varied practically together. As would be expected from the nitrification of progressively larger amounts of urea, the  $\text{NO}_3$  ions increased, and along with the accompanying decrease in pH, the  $\text{HCO}_3$  ions decreased.

## SUCROSE TREATMENTS

The absorption of nitrates attending the destruction of sucrose by micro-organisms can be considered the reverse process to that involved in the nitrification of urea by which the concentrations of nitrates can be built up in the

TABLE 5

*Effect of the biological destruction of sucrose on "nitrated" Fresno fine sandy loam as measured at various times during the incubation*

SUCROSE MGM -ATOMS CARBON per kilo	TIME OF INCUBATION WEEKS	1:10 = SOIL : H <sub>2</sub> O RATIO			QUIN pH AT 25°C. 1.1 SUSPENSION		N KCl EXTRACT AT pH 1 M.E. PER KILO	
		Mgm. in 20 ml.		Sp. cond.  Mhos × 10 <sup>-4</sup>	H <sub>2</sub> O	N KCl	NH <sub>4</sub>	NO <sub>3</sub>
		Susp'd solids	Soluble solids					
0	3/7	2.5	9.8	6.05	3.65	3.48	0.72	41.8*
	2	2.0	12.8	6.48	3.93	3.75	0.50	36.3
	4	0.8	12.9	6.67	4.26	4.09	1.58	37.7
	7	0.8	13.2	6.76	4.33	4.11	1.71	43.90
75	1	7.4	12.4	6.18	4.13	3.86	1.14	34.6
	2	8.5	11.2	6.03	4.47	4.28	2.56	33.3
	4	7.5	10.5	5.56	4.69	4.48	2.97	30.8
	7	3.6	11.3	6.38	4.60	4.52	4.01	41.42
	16	6.4	8.7	6.09	4.93	4.67	5.20	36.76
150	1	12.9	13.4	6.22	4.33	3.97	1.28	36.6
	2	11.0	10.8	5.95	4.63	4.42	2.79	31.9
	4	12.6	9.2	4.78	5.10	4.86	4.66	26.6
	7	6.7	10.5	5.82	4.96	4.89	6.94	38.61
	16	9.3	8.1	5.80	5.13	4.88	6.74	35.64
300	1	17.8	15.4	5.87	4.26	4.01	1.04	38.7
	2	18.8	9.0	5.25	5.29	5.08	2.68	28.4
	4	18.5	6.7	3.98	6.07	5.80	7.32	21.7
	7	12.2	8.8	5.33	5.46	5.30	9.14	33.35
	16	14.6	7.0	5.29	5.63	5.33	8.88	31.00
750	2	14.0	13.2	4.98	4.85	4.52	3.60	25.2
	4	20.5	6.5	3.24	7.23	6.99	10.34	12.34
	7	17.4	6.5	4.40	6.00	5.83	14.27	26.26

\* See footnote to table 1.

soil. We shall accordingly consider the indirect or dynamic effects of this reverse process upon the same soils.

The effects of the biological destruction of sucrose on "nitrated" Fresno fine sandy loam, as determined in these experiments at various times during the incubation period, are given in table 5. At all times during incubation there was increased dispersion with larger additions of sucrose. With the larger

applications, the amounts of suspended solids seemed to reach a maximum at about 4 weeks, the amount of dispersion diminishing after that time. The highest dispersion was obtained in the largest rate of sucrose treatment at 4 weeks of incubation. The amount, 20.5 mgm., was slightly more than 75 per cent of that obtained from the untreated control of the Fresno fine sandy loam, in the urea treatments of table 1. Here sucrose was able nearly to overcome the effects of the "nitrating" treatment given this soil.

TABLE 6

*The effect of the biological destruction of increasing amounts of sucrose on "nitrated" Fresno fine sandy loam after 4 weeks of incubation*

SUCROSE' MGM.-ATOMS OF CARBON PER KGM. SOIL . . . .	0	75	150	300	750
1:10—suspension:					
Susp'd. solids mgm. in 20 ml. . . . .	0.8	7.4	12.6	18.4	20.6
Extract—soluble solids. . . . .	12.9	10.5	9.2	6.7	6.4
Sp. cond. Mhos $\times 10^{-4}$ . . . . .	6.67	5.56	4.78	3.98	3.24
Quin pH 1:1 suspensions H <sub>2</sub> O . . . . .	4.26	4.69	5.10	6.07	7.23
N KCl. . . . .	4.09	4.48	4.86	5.80	6.99
Extract 1:1 = soil: H <sub>2</sub> O					
Sp. cond. Mhos $\times 10^{-4}$ . . . . .	47.7	43.3	35.7	29.6	19.6
Ions—m.e./kgm. soil:					
NH <sub>4</sub> . . . . .	1.08	1.82	2.90	4.53	6.34
K. . . . .	2.92	2.84	2.20	2.60	2.16
Na. . . . .	1.65	1.36	1.30	1.36	1.39
Ca. . . . .	30.72	26.44	20.02	13.93	5.83
Mg. . . . .	12.23	10.52	8.32	6.14	3.93
Total cations. . . . .	48.60	42.98	34.74	28.56	19.65
HCO <sub>3</sub> . . . . .	0.05	0.16	0.31	1.02	8.50
Cl. . . . .	0.73	0.73	0.67	0.76	0.64
NO <sub>3</sub> . . . . .	46.50	39.14	31.68	25.88	9.80
SO <sub>4</sub> . . . . .	0.93	0.77	0.61	0.47	0.19
PO <sub>4</sub> . . . . .	1.51	1.44	1.35	0.97	0.57
Total anions. . . . .	49.72	42.24	34.62	29.10	19.70

As the suspended solids increased, the specific conductance of the extract diminished to a minimum at about 4 weeks. This point also coincided with the time of the lowest amount of nitrates in each of the treatments. With the higher applications of sucrose, as the amount of nitrates again grew, the specific conductance increased and the pH decreased.

By comparing the first columns of Tables 2 and 6 the effect of "nitrating" the Fresno soil on the 1:1 extract may be noted. As should be expected, the concentration of basic cations increased greatly, bicarbonates decreased, and

nitrites increased markedly. Sulfates increased slightly, and soluble phosphates increased about 150 per cent.

The effects of the sucrose treatments on "nitrated" Fresno fine sandy loam after an incubation period of 4 weeks are shown more completely in table 6.

TABLE 7

*Effect of the biological destruction of sucrose on "nitrated" Yolo loam as measured at various times during the incubation*

SUCROSE MOM.-ATOMS CARBON PER KILO	TIME OF INCUBATION WEEKS	1:10 = SOIL:H <sub>2</sub> O RATIO			QUIN pH AT 25°C. 1:1 SUSPENSION		N KCl EXTRACT AT pH 1 M.E. PER KILO	
		Mgm. in 20 ml.		Sp. cond. Mhos × 10 <sup>-4</sup>	H <sub>2</sub> O	N KCl	NH <sub>4</sub>	NO <sub>3</sub>
		Susp'd solids	Soluble solids					
0	1	.....	7.8	3.88	6.00	5.45	0.94	25.0*
	4	1.1	5.1	3.78	6.11	5.47	1.64	22.3
	11	-0.4	5.8	4.52	6.07	5.28	1.00	29.44
50	1	0.9	7.2	3.54	6.21	5.58	0.99	22.2
	4	1.3	4.8	3.52	6.24	5.47	1.56	25.28
	11	0.3	5.3	3.92	6.09	5.37	0.64	25.22
100	1	4.5	6.7	3.22	6.40	5.70	1.58	20.4
	2	3.9	5.3	3.15	6.50	5.71	2.28	19.56
	4	3.7	4.2	3.31	6.29	5.58	2.24	19.52
	7	4.7	3.8	3.22	6.41	5.60	2.20	18.62
	11	0.8	4.8	3.52	6.12	5.40	0.76	22.00
200	1	15.2	6.4	2.96	6.71	5.99	2.04	16.9
	2	11.1	5.0	2.64	6.80	5.99	2.96	15.66
	4	8.6	4.1	2.90	6.58	5.80	3.68	16.24
	7	7.9	3.3	2.94	6.57	5.70	2.72	16.62
	11	1.5	4.5	3.33	6.19	5.42	0.52	20.62
400	1	17.8	6.8	2.86	6.97	6.21	2.34	13.5
	2	30.6	4.4	2.32	7.32	6.41	4.82	11.74
	4	27.6	3.6	2.50	7.04	6.17	5.84	11.48
	7	17.2	3.2	2.57	6.80	5.89	3.24	13.94
	11	5.7	3.9	2.84	6.45	5.70	2.00	16.60
750	2	27.1	6.1	2.42	7.29	6.48	3.02	9.42
	4	117.3	3.2	1.84	7.51	6.63	7.60	3.80
	7	49.2	3.0	2.22	7.25	6.31	4.24	9.80

\* See footnote to table 1.

These analyses were made at the time of the highest amount of suspended solids where the largest amount of sucrose had been added. Coincident with this high dispersion, the specific conductance of the extract was at a minimum. The N KCl extract at pH 1 showed a high amount of ammonia, which was also reflected by appreciable amounts in the water extract.



In the 1:1 extract the concentration of  $\text{NH}_4$  ions rose from 1.08 m.e. in the control to 6.34 in the case of the largest sucrose treatment. Potassium and Na ions in general declined, though some irregularities occurred. Calcium and Mg ions diminished progressively, the change of the former being much more marked than that of the latter. Among the anions, the  $\text{HCO}_3$  ions increased along with the pH of the suspensions. The  $\text{NO}_3$ ,  $\text{SO}_4$ , and  $\text{PO}_4$  ions diminished progressively and materially. The Cl ions varied slightly in concentration.

The number of the equivalents of the Ca, Mg, and  $\text{NO}_3$  ions, which were those of greatest numerical fluctuation, changed at about the same rate with increased sucrose treatment. Potassium and Na ions, as was found also in the nitrification experiments, changed less abruptly. With this soil, as the amount of sucrose increased, the concentration of  $\text{PO}_4$  ions in the 1:1 extract decreased. Although some of this may have been due to a chemical precipitation following the higher pH values attained, still a considerable amount of the disappearance of the phosphates may have been due to absorption by microorganisms. In this connection, Thompson *et al.* (42) have shown phosphate diminution in soil cultures of *Azotobacter chroococcum* when dextrose was added.

As with the urea treatments, the Yolo loam gave a picture somewhat different from that of the Fresno soil with respect to the results of the sucrose treatments. The effects of the biological destruction of sucrose upon the "nitrated" Yolo soil are given in table 7. With regard to suspended solids, the highest amount of sucrose added after 4 weeks of incubation caused the dispersion of the colloids, so that 117.3 mgm. remained in suspension after 24 hours. This was higher than the Yolo loam (cf. urea control table 3) had at first, namely, after 1 week's incubation.

With the treatment of 100 m.a. of carbon as sucrose, and larger applications also, the pH rose to a maximum and subsequently declined. In each case, this maximum pH occurred at the same time as the lowest specific conductance, and in the case of the two largest applications of sucrose, the maximum pH was coincident with the smallest amount of nitrate and with fairly large, if not the largest, amount of ammonia.

Of the more complete analyses, one set was made after 4 weeks of incubation and the other after 11 weeks. The one after 4 weeks is shown in table 8. Here the electrolytes were withdrawn sufficiently in the 750 m.a. of carbon added as sucrose to disperse the soil colloids to the highest value obtained in all the tests. The sucrose treatment had been able to overcome completely the flocculating effects of the  $\text{HNO}_3$  added. Undoubtedly, this was aided by the higher pH obtained with the  $\text{NH}_3$  liberated from the decomposition of the microbial protein. Potassium ions decreased except in the case of the 100 m.a. treatment, and Na ions progressively decreased with added sucrose, also. Calcium and Mg ions diminished markedly. Among the anions,  $\text{HCO}_3$  ions increased materially, and the concentration of the chloride ions rose also. Sulfate ions decreased progressively, but  $\text{PO}_4$  ions showed no consistent action.

Between the fourth week (cf. table 8) and the eleventh week (cf. table 9) the nitrification of some of the microbial proteins and similar compounds previously formed, occurred as shown by the considerable increase in nitrates during that time. Potassium and Na ions showed some differences, but they were hardly consistent with any easy explanation. With the larger sucrose applications Ca and Mg ions rose in concentration in the period following the

TABLE 8

*Effect of the biological destruction of increasing amounts of sucrose upon "nitrated" Yolo loam after 4 weeks of incubation*

SUCROSE: MGM. ATOMS OF CARBON PER KGM. SOIL . . . . .	0	50	100	200	400	750
1:10—suspension:						
Susp'd solids mgm. in 20 ml . . . . .	1 1	1 4	3.7	8.6	27 6	117.3
Extract soluble solids . . . . .	5.1	4 8	4 2	4 1	3 6	3 2
Sp. cond. Mhos $\times 10^{-4}$ . . . . .	3.78	3 52	3 31	2.90	2 50	1.84
Quin pH 1:1 suspensions $H_2O$ . . . . .	6 11	6 24	6 29	6 58	7 04	7.51
N KCl . . . . .	5 47	5 47	5 58	5 80	6 17	6 63
Extract 1:1 = soil: $H_2O$ :						
Sp. cond. Mhos $\times 10^{-4}$ . . . . .	28 60	24.45	24 20	17 88	15 41	6.65
Ions m e./kgm. soil:						
NH <sub>4</sub> . . . . .	0 48	..	0 44	0 56	0 72	0 81
K . . . . .	0 77	..	0 93	0.57	0.61	0 36
Na . . . . .	1 50	..	1 42	1.26	1.21	1 01
Ca . . . . .	9.82	..	8 65	5.52	4 51	1.90
Mg . . . . .	14 96	..	11 86	8 68	7 23	3.16
Total cations . . . . .	27.53	..	23 30	16 59	14.28	7.24
HCO <sub>3</sub> . . . . .	0 24	0 34	0 40	0 52	1.24	3.96
Cl . . . . .	0 22	0 22	0 28	0 34	0.45	0.73
NO <sub>3</sub> . . . . .	26.10	..	21 60	14 80	11 90	2.60
SO <sub>4</sub> . . . . .	0 40	..	0 30	0.21	0.13	0.09
PO <sub>4</sub> . . . . .	0 04	..	0 02	0 04	0.03	0.06
Total anions . . . . .	27 00	..	22 60	15 91	13.75	7.44
SiO <sub>2</sub> p.p.m. . . . .	58	..	50	40	35	22

first weeks of incubation. Among the anions, the bicarbonates diminished, whereas sulfates and nitrates increased during this period.

It is interesting to note that as nitrates diminished, presumably to form proteins in the microorganisms, sulfates also declined in amount (cf. table 8). These may be presumed to have also gone into the formation of proteins. The increases of both nitrates and sulfates between the fourth and eleventh weeks was presumably brought about by the decomposition of proteins previously formed. Again the equivalents of Ca, Mg, and NO<sub>3</sub> ions responded to

change at about the same relative rate, whereas K and Na ions reacted much more slowly.

Results similar to those reported herein, with the possible exception of those for  $\text{SO}_4$  ions, were obtained by Stewart (41) and by Burd and Martin (11, 12) in studying the effect of the growth of higher plants on soils. This similarity

TABLE 9

*Effect of the biological destruction of increasing amounts of sucrose upon "nitrated" Yolo loam after 11 weeks of incubation*

SUCROSE: MGM.-ATOMS OF CARBON PER KGM. SOIL.....	0	50	100	200	400
1:10 suspension:					
Susp'd solids mgm. in 20 ml .....	-0.4	0.3	0.8	1.6	5.7
Extract—soluble solids.....	5.8	5.3	4.8	4.5	3.9
Sp. cond. Mhos $\times 10^{-4}$ .....	4.52	3.92	3.52	3.33	2.84
Quin. pH 1:1 suspensions $\text{H}_2\text{O}$ .....	6.07	6.09	6.12	6.19	6.45
N KCl.....	5.28	5.37	5.40	5.42	5.70
Extract—1:1 = soil: $\text{H}_2\text{O}$ :					
Sp. cond. Mhos $\times 10^{-4}$ .....	31.42	27.21	25.78	24.22	20.21
Ions m.e./kgm. soil:					
NH <sub>4</sub> .....	0.15	0.10	0.06	0.12	0.30
K.....	0.76	0.69	0.61	0.61	0.52
Na.....	1.46	1.37	1.38	1.44	1.38
Ca.....	12.60	12.19	9.00	8.55	6.45
Mg.....	15.52	12.53	11.66	11.26	9.88
Total cations .....	30.49	26.88	22.71	21.98	18.53
HCO <sub>3</sub> .....	0.36	0.36	0.36	0.40	0.55
Cl.....	0.20	0.20	0.14	0.14	0.14
NO <sub>3</sub> .....	29.60	25.40	23.50	22.34	17.78
SO <sub>4</sub> .....	0.49	0.39	0.35	0.29	0.24
PO <sub>4</sub> .....	0.07	0.06	0.06	0.06	0.06
Total anions.....	30.72	26.41	24.41	23.23	18.77
SiO <sub>2</sub> p.p.m.....	56	54	56	51	46

of behavior was not unexpected in view of the fact that it was the growth of minute plants deriving their energy from the sucrose added which was responsible for the changes.

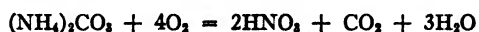
#### DISCUSSION

##### *Biochemical and cation—exchange reactions*

Urea in its decomposition goes through the following reaction:



The  $(\text{NH}_4)_2\text{CO}_3$  in solution partially reacts with the base exchange complex, and the pH of the medium goes up. The ammonium carbonate is nitrified according to the following reaction:



As the ammonium carbonate is oxidized to nitric acid, the hydrogen of the acid is dissociated as H ions. Brioux (9), Pierre (33), Jones (25) and the experiments reported herein showed the effect of urea decomposition on the reaction of soil; first the pH was raised, then it decreased to a value below that of the initial pH. Meanwhile, the H ions thus formed reacted with the replaceable cations of the exchange complex, giving a lower pH of the suspension and a higher specific conductance of the extract, and increasing concentrations of K, Na, Ca, and Mg ions in the soil solution. The theory connected with this phase of the problem has been explained in considerable detail by such workers as Pierre (33) and F. E. Allison (1).

With the exception of urea, most organic nitrogenous substances which are initially concerned in ammonification and nitrification are proteinaceous in nature. Pure proteins (essentially neutral in reaction) contain only carbon, hydrogen, oxygen, nitrogen, sulfur, and phosphorus. Any protein, on being fully oxidized with oxygen and its products dissolved in water, will yield 1 mole each of  $\text{H}_2\text{CO}_3$ ,  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{H}_3\text{PO}_4$  for each gram-atom respectively of carbon, nitrogen, sulfur, and phosphorus contained in the original protein. The effect of the  $\text{H}_2\text{CO}_3$  as an acid is variable, depending upon the properties of the system being considered. At low pH values the  $\text{H}_2\text{CO}_3$  is easily lost from the soil as  $\text{CO}_2$ , and that which remains is but weakly dissociated to form H ions. With increasing alkalinity its acidifying effect becomes more pronounced, but is here considered almost negligible for neutral and acid soils. With the exception of carbon, there are many more gram-atoms of nitrogen than of the other elements named in each of the proteins. Consequently, the nitrification of organic materials to form nitrates may be expected to dominate in soils where  $\text{H}_2\text{CO}_3$  has but little effect as an acid. Thus, we would expect results similar to those obtained with urea from other nitrogen-containing organic materials on oxidation to nitrates, with due allowance for the chemical action of the  $\text{H}_2\text{CO}_3$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{H}_3\text{PO}_4$  simultaneously produced. These acids must result from the complete oxidation of these organic materials whether the processes take place by purely chemical means in the laboratory or biologically in the soil. The writer has developed this theory more fully elsewhere (14).

With larger applications of sucrose, undoubtedly the most important contributing cause of the increase in pH was what is known to plant physiologists as "physiological alkalinity" currently explained by a more rapid absorption of  $\text{NO}_3$  ions than of the basic cations in the soil solution, but more clearly explained by Pantanelli (32) and also more recently by F. E. Allison (1) by the absorption of  $\text{HNO}_3$  out of the soil solution. Pantanelli postulated the absorption of H

ions with the  $\text{NO}_3$  ions leaving the  $\text{OH}$  ions with the cations with which the  $\text{NO}_3$  ions were originally paired. In studies involving the absorption of ions with seedling plants, Hoagland (21) suggested the replacement of  $\text{NO}_3$  ions with  $\text{HCO}_3$  ions by the plants. Practically, in a system in which  $\text{CO}_2$  was present in sufficient amounts, the results would not be different from those proposed by Pantanelli and Allison as



If the soil organisms absorb nitrates largely as  $\text{HNO}_3$ , we would expect the reversal of the conditions we found with the nitrification of urea. As  $\text{HNO}_3$  was formed from urea, a large part of the H ions thus made available replaced the basic cations in the exchange complex. With the removal of  $\text{HNO}_3$  by the microorganisms the basic cations went back into the places formerly occupied by them.

Work already cited suggests the absorption of nitrates as  $\text{HNO}_3$ . It can be shown even more definitely by a line of theoretical reasoning that it is in the form of  $\text{HNO}_3$  that plants use nitrates in producing pure proteins. As stated in the foregoing, pure proteins, on complete oxidation with oxygen give 1 mole each of  $\text{H}_2\text{CO}_3$ ,  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{H}_3\text{PO}_4$  for each gram-atom of carbon, nitrogen, sulfur, and phosphorus, respectively, contained in them. In the production of the same pure proteins, plants must, therefore, use 1 mole each of  $\text{H}_2\text{CO}_3$  (or  $\text{CO}_2$ ),  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{H}_3\text{PO}_4$  for each gram-atom of carbon, nitrogen, sulfur, and phosphorus<sup>3</sup> respectively, occurring in the proteins thus formed where these elements are furnished as bicarbonates (or  $\text{CO}_2$ ), nitrates, sulfates, and phosphates. Other nitrogenous organic compounds occur in plants. But as proteins are the dominant ones, their formation is undoubtedly the dominant one in the utilization of nitrates by plants. It is entirely probable that with the production of many other organic compounds containing carbon, nitrogen, sulfur, and phosphorus in plants (both higher and lower forms),  $\text{H}_2\text{CO}_3$  (or  $\text{CO}_2$ ),  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{H}_3\text{PO}_4$  are the fully oxidized forms of the respective elements which are used.

Nitrates decreased in the soil solution when sucrose was added in our experiments. It might be argued that the basic cations, occurring in the soil solution with them were absorbed by the microorganisms at the same time. It is true that a neutral salt added to a soil will lower the pH, and that the withdrawal of a neutral salt should likewise increase the pH. With the Fresno soil (cf. control table 1), at 3 days of incubation, the water suspension gave a pH of 7.33, whereas the *N* KCl suspension gave a pH of 6.36, a pH change of approximately 1 unit. At other times, the pH change with KCl added was much less. Here the addition was of 1,000 m.e. of KCl per kgm. In the case of the

<sup>3</sup> Phosphorus, according to Haas and Hill (18), probably does not occur in plant proteins, but is here included as a theoretical possibility.

<sup>4</sup> Generally only two of these three fully oxidized forms would be utilized in the formation of any one substance by a plant not capable of photosynthesis.

"nitrated" Fresno soil, the withdrawal of nitrates was a little less than 40 m.e. per kgm. This amount of withdrawal is far too small to account for a pH change of 3 units.

Unless we assume a marked alteration in the exchange complex of this soil, we must admit that H ions left it and basic cations entered it. Otherwise, there would have been no such change in the pH of the 1:1 aqueous suspension. It seems, therefore, probable that a very considerable part of the basic cations disappearing from the soil solution re-entered the exchange complex as predicated by F. E. Allison (1).

It is recognized that when organic materials are broken down to purely inorganic compounds, the cations which appear in the soil solution do not come from the exchange complex alone. Undoubtedly, a considerable proportion of these was present within the organic materials at the start of its decomposition. Similarly, all of the cations which disappeared from the soil solution due to the withdrawal of H ions did not enter the exchange complex. Some undoubtedly were absorbed by the organisms themselves.

Another biological factor causing a change in the pH was an increasing amount of  $\text{NH}_4$  ions among the replaceable cations as disclosed by the *N* KCl extract at pH 1. Especially with the Fresno soil, a soil in which nitrification was delayed in the urea treatments, a considerable amount of  $\text{NH}_4$  ions accumulated. As, obviously, the principal source of the ammonia was the nitrate occurring in the soil at the beginning, the transformation from  $\text{NO}_3$  to  $\text{NH}_4$  ions, or rather the change from  $\text{HNO}_3$  to  $\text{NH}_4\text{OH}$ , was the cause of the increase in pH as far as this factor is concerned.

Although the chain of processes by which  $\text{NH}_4$  ions increased in the exchange complex was of small moment in determining the exact pH at any one time, it is necessary to know the processes by which the ions arose, in order to appreciate what to expect with a continuation of them. With an increase of  $\text{NH}_4$  ions before the  $\text{NO}_3$  ions stopped decreasing, the question comes up as to the direct origin of the ammonia. Was it formed directly from the nitrate or was it formed indirectly by deaminization of amino compounds which in turn had been formed from the nitrates? Kostytschew and Tswetkova (30) showed that ammonia is one of the stages between nitrates and amino compounds.

In our experiments (cf. tables 5 and 7), ammonia certainly began to appear at the end of 1 week, whereas in the larger rates of sucrose application the lowest point of nitrate concentration was not reached until the fourth week. This sequence of events did not preclude the possibility of ammonia having arisen by the deaminization of microbial proteins and similar compounds already formed from the original nitrates. It is natural to suppose that this was the dominant process after the nitrates began to increase. It could very well have been the dominant process also as nitrates were diminishing, simply by the deaminization of compounds (in the old microbial cells) already formed. Some other evidence is applicable here. Jensen (24) showed that dried fungous bodies as well as dried growth of *Actinomyces*, when added to sand, produced

considerable quantities of ammonia in 10 days. Our data give no inkling as to whether some of the ammonia first appearing was directly formed from nitrate or secondarily from amino compounds.

A third factor was operative in the change of pH. The control "nitrated" soils which received no sucrose (cf. tables 5 and 7) gradually increased in pH values during the course of incubation. This is especially marked with the Fresno soil (cf. table 5) where the pH in the aqueous suspensions increased from 3.65 after 3 days to 4.33 after 7 weeks of incubation. This increase might have been due to a slow exchange of basic cations of the inner layers of the soil colloidal crystals by the H ions from the soil solution or from those of the outer layers of the soil colloids, namely, that part referred to as the "exchange complex." It might have been due, of course, to a change to a weaker kind of aluminosilicic acid, i.e., to a molecular rearrangement where H ions would be less dissociated than formerly.

### *Resultant effects on colloid dispersion*

Factors which undoubtedly cause changes in colloid dispersion in the soils used in these experiments include:

Variations in the composition and concentration of the electrolytes in the dispersion medium. Most of the changes in colloid dispersion which are not due to experimental error are attributed to this factor.

Variation in the amounts of each of the different cations in the exchange complex. The influence of the variation in the amount of H ions in the exchange complex on colloid dispersion is indicated by the work of Bradfield (7). He added various mixtures of KCl and KOH and found over 10 times as much electrolyte (as measured by the specific conductance of the dispersion medium) was required to flocculate his soil colloids at pH 8 as at pH 5.2. The dispersing action of ammonia on soil colloids follows the same general principle.

The deflocculating effect of ammonia formed through biological agencies in soils observed in these experiments (cf. table 1) has been noted elsewhere. Russell (36) warned against the use of liquid manure, especially in excessive amounts, on heavy soils because of the resulting deflocculation of the soil colloids and the formation of a crust on the soil surface in the field. Hager (19) has explained this deflocculation as due to  $(\text{NH}_4)_2\text{CO}_3$  formed from the decomposition of the nitrogenous compounds in the liquid manure. On soils which nitrify ammonia readily, he has stated that this unfavorable condition soon changes as the nitrates are formed from the ammonia.

Subject only to the ability of a soil, the colloids of which remain electro-negative, to form and retain nitrates from an adequate supply of organic materials, the soil solution must become concentrated enough with continual formation of these compounds to precipitate the colloids in a 1:10 aqueous suspension. This is what we find with the Yolo soil. If the Fresno soil had been able to nitrify urea completely with the higher concentrations of urea tried, this would have resulted in flocculation of the colloids, as the preliminary experiments not reported here showed that  $\text{HNO}_3$  of equivalent concentration

would do. This was also shown by the control "nitrated" Fresno soil in the sucrose treatments reported herein.

Given a system in which  $\text{HNO}_3$  artificially or biologically added causes flocculation of the soil colloids, it would seem natural that the effect of biological processes which would result in the withdrawal of  $\text{HNO}_3$  from the soil solution by microorganisms which are supplied with sucrose and similar materials would cause the dispersion of the soil colloids if continued long enough, unless by-products of these processes would keep them flocculated. In this regard, the question has been raised as to the possible effect of  $\text{CO}_2$  liberated by the decomposition of the sucrose. Judged by its dissociation constant,  $\text{H}_2\text{CO}_3$  is but slightly effective as an acid below a pH of 6, and with decreasing pH values its effectiveness becomes even less, so that at the pH of these experiments it would not be expected to exert much influence, especially when  $\text{CO}_2$  has an opportunity to escape. If these tests were conducted with soils at a higher pH, we might expect the  $\text{H}_2\text{CO}_3$  liberated to keep the soils flocculated. Thus, Breazeale (8) described an experiment where the suspension of a soil containing  $\text{CaCO}_3$  was flocculated by bubbling  $\text{CO}_2$  through it. At a pH of 8 or more  $\text{H}_2\text{CO}_3$  has an appreciably acid effect.

#### GENERAL DISCUSSION

The water-soluble substance, urea, produced changes in colloid dispersion correlated with the  $\text{HNO}_3$  produced by the organisms concerned, whereas the water-soluble sucrose produced changes correlated with the  $\text{HNO}_3$  withdrawn by the organisms.

In a like manner, applications of non water-soluble organic materials would be expected to produce like results in proportion to the nitrates produced or nitrates consumed by the organisms breaking them down, except in so far as these non water-soluble materials would influence the properties of the soil directly or statically.

In general, the chemical as well as the physical effects on soils here shown are best interpreted in a broad way, not on the basis of nitrates produced or consumed, but rather on the basis of the H ions appearing and disappearing in the soil system. Thus it was the H ions that became completely ionized as the nitric acid was produced from urea which were the active agents of the changes which followed. On the other hand, it was the withdrawal of H ions with the  $\text{NO}_3$  ions to form microbial proteins which carried these chemical and physical processes through in the opposite direction where sucrose and other materials inducing nitrate-disappearance were added.

Other experiments have shown the effects of H ions added or biologically produced upon the physical properties of soils. Stephenson and Powers (40) obtained increased flocculation of the soil colloids with larger applications of sulfur, which, as a result of its biological oxidation, yielded a large amount of H ions with the  $\text{SO}_4$  ions produced. Haynes (20) obtained an increased rate of percolation of water through black alkali soils after sulfur applications.



Kelley and his co-workers (28, 29) found improved penetration of irrigation water and a general improvement in tilth where sulfur, iron sulfate, and alum were applied in sufficient quantities to black-alkali soils. All three of these treatments caused H ions to increase in the soil.

Practices resulting in the increase of electrolytes in the soil, especially those furnishing divalent cations, have in many cases markedly improved the tilth of the soil. It would be expected that biological activities which would result in increased H-ion concentrations in soils whose exchange complex is well supplied with divalent cations would increase the latter in the soil solution, which in turn would cause a better physical condition of the soil. However, the experience of Sievers and Holtz (38) that the value of organic matter in improving the physical condition of the soil is proportional to its nitrogen content is best explained, not on the basis of the static rôle of the organic materials, but rather in part at least upon the amount of nitrates (therefore H ions) produced in its decay. The same explanation, which takes into account the possibility that the growth of higher plants may also be a means of reducing H ions by the absorption of nitrates, is suggested for Chapman's findings. Stephenson's procedure is so nearly like our own that in so far as our results check his, the same explanations are given for them. The explanation of his other observations must await further experimentation.

One purpose of this paper has been to draw attention to the purely dynamic rôle of organic substances in soils. The methods of procedure used and the water-soluble substances selected were such as to emphasize this function particularly. The results herein reported cannot, we believe, be explained on the basis of "static" organic matter. Until the dynamic rôle is definitely recognized, then, it cannot be differentiated from the static one. Though some of the functions of organic matter which have heretofore been ascribed to its static rôle may later be credited to its dynamic one, it is the opinion of the writer that both should be recognized.

#### SUMMARY

Organic materials may influence the physical condition of soils in two ways: *directly* by their purely physical properties, and *indirectly* by changes in the electrolyte concentration of the soil solution resulting from the utilization of these materials by microorganisms. The purpose of this paper is to present a study of this *indirect* effect.

The organic materials used were urea and sucrose—both non-colloidal, water-soluble, and practically free from electrolytic properties, and therefore of negligible direct effect on the soil colloids, either from a purely physical point of view or from a physico-chemical one.

The criterion of colloid dispersion was the amount of suspended solids (in a soil suspension whose soil-water ratio was generally 1 to 10) obtained according to a standardized procedure.

Ammonification of urea proceeded normally in both soils. Incomplete

nitrification of the largest applications of urea to one soil, Fresno fine sandy loam, resulted in large accumulations of ammonia, and in increases in pH and colloid dispersion. These results are attributed to the  $(\text{NH}_4)_2\text{CO}_3$  arising from ammonification of the urea. Normal nitrification of urea in the other soil, Yolo loam, caused decreases in pH and colloid dispersion. It also increased specific conductance and concentrations of nitrates and basic cations, especially Ca and Mg in the liquid phase. These effects are assignable to the  $\text{HNO}_3$  produced in the nitrification of the urea.

The application of sucrose to "nitrated" (the nitrates had previously been increased by the addition of  $\text{HNO}_3$ ) samples of both soils caused decreases in specific conductance, in cations, and in nitrate ions of the liquid phase, and increases in pH and colloid dispersion. These findings had their origin in the withdrawal of  $\text{HNO}_3$  from the liquid phase by microorganisms to produce microbial proteins. The basic cations moved meanwhile to the exchange complex to take the places formerly occupied by the H ions.

In the sucrose-treated soils  $\text{NH}_4$  ions began to increase early in the incubation period. Later, especially in the Yolo soil, nitrates, basic cations, and specific conductance increased in the liquid phase, while pH and colloid dispersion in general decreased. We account for these facts by the breakdown of the microbial proteins formed, and the conversion of the nitrogen first to ammonium carbonate and subsequently to  $\text{HNO}_3$ —a reversal of the processes taking place early in the incubation of the sucrose.

During incubation the "nitrated" control (no additions of sucrose) samples slowly increased in pH, presumably in adjustment to the  $\text{HNO}_3$  added in "nitrating."

Other investigators have shown that some of the same effects observed in this study, namely, decreased dispersion and increased rate of percolation accompanying the increase of nitrates, have also been obtained with other H-ion producing amendments (sulfur, iron sulfate, alum, etc.). Often marked improvements in soil tilth were noted in the field as results of these amendments. May the improvement in soil tilth following the application of some organic materials not in part result from the formation of acids ( $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{H}_2\text{CO}_3$ ,  $\text{H}_3\text{PO}_4$ , etc.) arising from the utilization of these organic substances by microorganisms?

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# THE INFLUENCE OF THE pH OF A CULTURE SOLUTION ON THE RATES OF ABSORPTION OF AMMONIUM AND NITRATE NITROGEN BY THE TOMATO PLANT<sup>1</sup>

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From the scientific standpoint, the presence of nitrogen in the form of a cation ( $\text{NH}_4^+$ ) in one case, and in the form of an anion ( $\text{NO}_3^-$ ) in the other, leads to theoretical implications which are manifold. From the practical standpoint, the importance of a thorough knowledge of the course of absorption and assimilation of various forms of nitrogen by plants is apparent from a consideration of the huge quantities of nitrogen used in fertilizers every year. Furthermore, the development of synthetic nitrogen fertilizers increases the practical possibilities.

The question of the relative efficiency of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  as sources of nitrogen for green plants is one which has often been investigated since the days of Liebig, the great agricultural chemist. Hutchinson and Miller (14, 15), Prianischnikow (33), and Mevius (25) have reviewed the early experiments which proved that plants could absorb and assimilate either  $\text{NH}_4\text{-N}$  or  $\text{NO}_3\text{-N}$ . However, considerable disagreement has been evident concerning the relative efficiency of these two forms of nitrogen, and the controversy apparently is not yet closed.

## *On the relation of the pH of the culture solution to the absorption of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$*

It is significant that Maze (22, 23), who used sterile solution culture methods, obtained yields of maize when  $(\text{NH}_4)_2\text{SO}_4$  was used as the source of nitrogen, comparable with those produced when  $\text{NaNO}_3$  was employed as the source of nitrogen, provided that  $\text{CaCO}_3$  was present in the culture solutions and that the concentration of  $(\text{NH}_4)_2\text{SO}_4$  did not exceed 0.5 parts in 1,000. The effect of adding the  $\text{CaCO}_3$  was probably to raise the pH of the solution, an effect which in turn influenced the absorption or assimilation, or both, of  $\text{NH}_4\text{-N}$  by the plants. Maze (23) analyzed the solutions in which his plants had been grown for many days and demonstrated that  $\text{NH}_4\text{-N}$  had been absorbed by the plants.

Pantaneli (30) studied the relative rates of absorption of the respective anions and cations of many salts by placing plants in these single-salt solutions for absorption intervals of 8 hours. Curiously enough, he reported no absorption of  $\text{NH}_4\text{-N}$  by *Vicia faba* from a solution of  $\text{NH}_4\text{NO}_3$ . In contrast, Prianischnikow (33), who used absorption intervals of 2 hours and a solution of  $\text{NH}_4\text{NO}_3$ , reported no absorption of  $\text{NO}_3\text{-N}$  by maize in most cases, but considerable absorption of  $\text{NH}_4\text{-N}$ .

Hoagland (13) presented data showing that the absorption of several anions, including  $\text{NO}_3^-$ , was greater from a solution of acid reaction (pH 5 to pH 5.5) than from a solution with a neutral reaction (pH 6.8). The plants were grown in the solution for 9 days, after which analyses of the solutions were made.

Theron (43) analyzed solutions in which plants had grown for 3 or 4 days and concluded

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<sup>1</sup> Journal Series paper of the New Jersey Agricultural Experiment Station, department of plant physiology.

that the influence of the reaction was most marked on the rates of absorption of the cations. Invariably, more cations were absorbed from alkaline than from acid solutions. He also noted some effect of the reaction of the solutions on the absorption of  $\text{NO}_3\text{-N}$ , but the effect was not so pronounced as that on the cations and was opposite to the effect upon the cations, more anions being absorbed from acid than from alkaline solutions.

Mevius and Engel (26) reported an increased rate of absorption of  $\text{NH}_4\text{-N}$  by maize from a culture solution as the pH of the solution was increased from pH 4.3 to pH 6.1, or from pH 6.1 to pH 7.1.

Sabinin and Kolotova (38) analyzed juices from cut stumps of plants grown in culture solutions and also analyzed the solutions. They concluded that at the acid reaction (pH 4.0 to pH 5.0) anions enter the plant in greater quantities than do cations, and that at the alkaline reaction (pH 7 to pH 8) cations enter more rapidly than the anions.

Niklewski, Krouse, and Lemanczyk (28) worked with single-salt solutions to which a buffer had been added. They noted that the alteration of the pH of the solution affected the absorption of the ions of a salt in a pronounced manner, although the presence of the buffer in some solutions introduced a complication by its effect on the roots of the plants.

Loo (20) allowed corn seedlings to grow for periods of 24 hours in solutions and determined that the amount of  $\text{NH}_4\text{-N}$  absorbed from the solutions increased with an increase in alkalinity and that the amount of  $\text{NO}_3\text{-N}$  absorbed was greatest at a weakly acid reaction.

Naftel (27) grew cotton in culture solutions and placed the plants in fresh solutions for absorption tests of 24 hours. He concluded that  $\text{NH}_4\text{-N}$  was used in larger amounts than  $\text{NO}_3\text{-N}$  by young seedlings until they were from 3 to 5 weeks old, and that after this stage, more  $\text{NO}_3\text{-N}$  was absorbed than  $\text{NH}_4\text{-N}$ . He also observed that  $\text{NH}_4\text{-N}$  absorption increased as the acidity of the culture solution decreased, and that the absorption of  $\text{NO}_3\text{-N}$  was only slightly affected by the reaction of the solution used.

Pirschle and Mengdehl (32) made extensive tests of the absorption of ions by peas, but did not study the effect of the pH of the solutions on the rates of absorption.

Prince, Jones, and Shive (35) studied the absorption of ions by soybeans from a four-salt solution in which the plants were grown. They analyzed the experimental solutions after successive 4-hour absorption intervals and at different stages in the life history of the plants. They found that the plants drew rather heavily upon the  $\text{NH}_4\text{-N}$  during the early stages of growth, and less heavily upon the  $\text{NO}_3\text{-N}$ , but that during the later stages of development, this condition was reversed. Jones and Skinner (17) reported similar data with maize plants and also showed that the concentration of  $\text{NO}_3\text{-N}$  in the culture solution affected the absorption of  $\text{NO}_3\text{-N}$ .

Using an improved technique for testing absorption, Stahl and Shive (41, 42) have shown that not only the age of the plant, but also the type of plant itself influences the rates of absorption of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ .

To generalize from the aforementioned experiments: it has been shown that the pH of the solution may affect the rate of absorption of both  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  by plants, although not all of the investigators have agreed on both of the ions. In most cases, however, it has been observed that a relatively low pH of the culture solution favored a higher rate of absorption of  $\text{NO}_3\text{-N}$  than did a high pH; whereas a relatively high pH of the culture medium resulted in a higher rate of absorption of  $\text{NH}_4\text{-N}$  than did a low pH. Furthermore, it has been shown that when both ions are present in the solution, the relative rates of absorption of the two are influenced by the pH of the medium, by the concentration of the ions in question in the solution, by the age of the plant, and by the species used as indicator plants.

The main purposes of the research described in the following pages were: (a) to study the relative rates of absorption of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  from a culture solution in which these two forms were present in approximately equal proportions simultaneously; (b) to study the influence of the pH of this culture solu-

tion upon the rates of absorption of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  by the plant; (c) to study the influence of the age of the plant upon these relative absorption rates; and (d) to note any possible relationship between these absorption rates and the concentrations of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  in the plant tissues. No attempt is here made to consider nitrogen absorption rates as determined systematically by experimental testing in relation to assimilation of nitrogen by the plant, nor in relation to the distribution of the nitrogen fractions in the plant tissues. This feature of the study will be reserved for a later publication.

The tomato plant (*Lycopersicum esculentum*, Mill.) was selected for these investigations because its vegetative development extends over a relatively long period of time during the life cycle, and because it has been shown (44) that its chemical composition is influenced by the pH of the culture solution as well as by the source of nitrogen in the culture medium.

#### METHODS OF EXPERIMENTATION

##### *Method of growing plants*

The seeds, selected<sup>2</sup> from a single plant of the Marglobe variety, were germinated in washed white sand which was kept moist with tap water. One week after germination, the seedlings were removed from the sand, washed free of adhering particles, and placed in a culture solution in 2-quart, colorless glass fruit jars. Three seedlings were fixed in a paraffined cork stopper and suspended in each jar by a method similar to that described by Tottingham (45). White cardboard covers around the culture vessels excluded most of the light from the roots.

The solution used was one of the modified Tottingham solutions described by Jones and Shive (18). Solution  $\text{T}_3\text{R}_2\text{C}_2$  of the Jones-Shive series was selected because it contained approximately equal molar concentrations of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  and had, in preliminary tests, produced excellent growth of tomatoes. This solution was modified by including only one-third of the amount of  $\text{KH}_2\text{PO}_4$  called for. The  $\text{KH}_2\text{PO}_4$  concentration was decreased in order to avoid precipitation, which occurred in the pH 7 solution when the original formula was used. The composition of the modified solution, which had a total osmotic concentration of 0.8 atmosphere, is given in table 1, together with the quantities of acid or alkali added to produce the desired pH.

In addition to the elements listed in table 1, traces of boron, manganese, and iron were added to the solution. Boron in the form of  $\text{H}_3\text{BO}_3$  and manganese in the form of  $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$  were added in quantities sufficient to give a concentration of 0.5 p.p.m. of each of these elements. Iron in the form of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  was added in quantities necessary to prevent chlorosis of the plants from iron deficiency. Concentrations ranging from 0.5 to 4 p.p.m. were used.

<sup>2</sup> The seeds were obtained from Dr. W. R. Robbins of the New Jersey Agricultural Experiment Station, who had made the selection.



The solution first supplied to the plants was prepared in 5-gallon bottles and adjusted to pH 5.5 with *N* KOH. Only one-half the concentrations given in table 1 were used while the plants were small. The solutions were renewed twice after successive 7-day intervals, once after a succeeding 4-day interval, and once after a final 2-day interval before the plants were placed in solutions of different pH.

After 20 days the plants were transferred from the solution in which they had been previously grown, to the solutions adjusted to pH 4.0, pH 5.0, pH 6.0, and pH 7.0. Five cultures, each including three plants, were grown at each pH level. Nine extra cultures were grown at both pH 4 and pH 7 for transfers to be made later.

At the same time, continuous solution renewal and continuous aeration of all the solutions were started. The method of continuous solution renewal was

TABLE 1  
*Composition of solutions used*

Basic solution				
SOLUTION NUMBER	PARTIAL VOLUME-MOLECULAR CONCENTRATIONS			
$T_2R_2C_2$ (modified) Tott.	$KH_2PO_4$	$Ca(NO_3)_2$	$MgSO_4$	$(NH_4)_2SO_4$
	.00211	.00292	.00711	.0028
Modifications				
QUANTITY	ACID OR ALKALI PER LITER		RESULTING pH	
cc.				
0.14	Normal $H_2SO_4$		4.0	
0.08	Normal KOH		5.0	
0.84	Normal KOH		6.0	
2.25	Normal KOH		7.0	

that devised by Shive and Stahl (40), and the method of aeration was that used by Clark and Shive (8) previously described by Allison (1). The importance of continuous solution renewal and continuous aeration of the culture solutions in producing vigorous plants for experimentation can not be overemphasized. Shive and Stahl (40) have mentioned several experiments showing the need of continuous solution renewal. In this experiment, continuous renewal was necessary, not only to maintain a more nearly constant pH, but also to maintain a relatively constant concentration of  $NH_4$ -N and  $NO_3$ -N in the solutions in replicate culture vessels. The beneficial effects of continuous aeration on root development and top growth in tomatoes in similar solutions have been shown by Clark and Shive (8).

The initial rate of continuous solution renewal was 1 liter in 24 hours. This was increased to 2 liters in 24 hours 5 days before the first absorption test was

made. This rate was further increased to 2 liters in 14 hours 5 days before the second adsorption test was made.

*Method of determining absorption rates*

The details of the absorption tests will be described in connection with the consideration of the results reported for each test, because certain changes in technique were necessitated with increase in age and consequent size of the plants. The absorption intervals were 6 hours in duration and covered a period of from 9 a.m. to 3 p.m. The three plants included in each culture, together with the cork stopper supporting them, were removed from the culture vessel in which the plants had been growing, the roots of the plants were

TABLE 2

*Changes in pH of the culture solution brought about by plants 41 days old*

ORIGINAL pH OF SOLUTION IN RESERVOIRS	pH OF SOLUTIONS FROM OUTLETS OF CULTURE VESSELS BEFORE ABSORPTION TEST	FINAL pH OF SOLUTIONS USED FOR ABSORPTION TEST
4.0	4.2-4.4	5.05-5.2
5.0	4.7-4.8	5.3-5.6
6.0	5.5-5.8	6.0-6.2
7.0	5.9-6.0	6.2

TABLE 3

*Rates of absorption of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  by plants 41 days old*

pH OF CULTURE SOLUTION	AVERAGE GREEN WEIGHT PER PLANT	N ABSORBED PER 100 GM. GREEN TISSUE IN 6 HOURS		
		$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$	Total
	gm.	mgm.	mgm.	mgm.
4.0	53.0	3.4	4.8	8.2
5.0	55.1	4.2	5.9	10.1
6.0	64.6	4.6	4.1	8.7
7.0	57.8	6.6	3.0	9.6

rinsed with a very fine stream of distilled water for a definite period of time, and the plants were then placed in the fresh solution in the test jar.

Enough solution was used for the test so that continuous renewal at the same rate previously maintained was possible. This continuous renewal of solution during an absorption test has been shown to be necessary by Stahl and Shive (41). At the end of the test interval the plants were removed from the solution and the roots were again rinsed. This wash water, the solution in the culture vessel, and any solution left in the reservoir of the renewal apparatus, and that in the beaker containing the overflow from the culture vessel, were all collected and diluted to the original volume. Analyses of these solutions showed the amount of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  removed from the original solution during the absorption interval. The plants used for the absorption test were returned,

at the expiration of the test interval, to the solutions in which they had been growing, were harvested within a few hours, and were immediately prepared for analyses of the tissues.

The  $\text{NH}_4\text{-N}$  in the test solutions was determined by the method of Sessions and Shive (39) with a modification which involved substituting 0.125 *N* sodium hydroxide for the sodium carbonate originally suggested. The  $\text{NO}_3\text{-N}$  in the test solutions was also determined by the method of Sessions and Shive (39).

### *Method of determining pH*

In making pH determinations of the culture solutions, a series of indicators prepared according to directions given by Clark (9) were used. Brom phenol blue, methyl red, brom cresol green, chlor phenol red, and brom thymol blue were selected because they measured a range of pH values from 3.8 to 7.4. The pH of each solution was estimated to within 0.05 of a pH unit by comparing the color developed by the addition of the required indicator with the colors of a set of LaMotte permanent color standards. The values thus obtained were occasionally checked against those of the same samples determined electrometrically by means of the hydrogen electrode and the type "K" potentiometer.

## EXPERIMENTAL RESULTS AND DISCUSSION

### *Results with plants 41 days old*

*Experimental procedure.*—The first absorption test was made after all the plants had been grown for a period of 14 days in the respective solutions of pH 4.0, 5.0, 6.0, and 7.0 in which they had first been placed. Continuous renewal of the culture solutions had proceeded at a rate of 1 liter in 24 hours until 5 days before the absorption test. At that time the rate of renewal was increased to 2 liters in 24 hours, in order to maintain a more nearly constant pH in the solution in which the plants were growing.

To show the necessity for rapid and continuous renewal of the culture solutions, the data in table 2 are presented. The solutions from the outlets of the culture vessels were collected one-half hour before the plants were transferred to the test solutions. The pH of the sample solution collected is representative of the pH of the solution in the corresponding culture jar, which was well mixed by the aerating system. The final pH of each solution used for the absorption test represents the pH of the solution after it had been diluted to the original volume at the completion of the test.

These data show the marked changes in reaction produced in the culture solutions by the plants, even with continuous renewal proceeding at a rate of 2 liters per day. The table indicates, however, that the changes in pH were rather well defined within narrow limits for replicate cultures grown in any one of the solutions.

Continuous renewal and aeration of the solutions were discontinued for 15 to 60 minutes before the plants were transferred to the fresh solutions for the

absorption tests. One liter of solution having the same composition as that in which the plants had previously been grown was used as the test solution for each culture. Of this solution 450 cc. was placed in a pint jar, in which the roots of the plants were suspended during the absorption interval of 6 hours. The remaining 550 cc. of the solution were dripped through the culture jar during the test interval so that the rate of renewal during the test interval was approximately the same as that preceding the test. It was not practicable to aerate the cultures during the absorption test interval, however. With this exception, the environmental conditions during the absorption test were approximately the same as those preceding the test. Hence, it is reasonable to assume that the rates of absorption of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  during the 6-hour test interval must have closely approximated the corresponding rates prevailing just before the plants were transferred to the fresh solutions.

The absorption of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  by these plants during the 6-hour interval, as well as the green weights of the plants, are recorded in table 3. The values of the average green weight per plant were calculated from the plants in five replicate cultures, each of which included three plants. Thus each value in the table represents the average for 15 plants. The absorption values are the averages for three of these five cultures, only the three most uniform cultures having been selected for the absorption test. The plants from all five cultures were then harvested and this material was used in the chemical analyses. In order to provide a standard for comparison, the average absorption rates are expressed in milligrams of nitrogen absorbed per 100 gm. of green tissue. The absorption rates per unit of green tissue were calculated separately for each culture, and the averages of the three cultures tested in each solution of the different pH values appear in table 3. To coincide with the aliquots taken for analysis, 100 gm. was chosen as the unit of green plant tissue.

*Appearance of plants.*—Table 3 shows that those plants grown in the pH 6 solution were the largest at this particular age. Although the differences in average green weight per plant between the plants grown in the pH 6 solution and those grown in the pH 4, pH 5 and pH 7 solutions are sufficiently large to be significant, the differences in average green weight per plant between the plants grown in the solutions at pH 4, pH 5, and pH 7 are probably not sufficiently large to be significant. All the plants were very vigorous and the tops were so much alike that no pronounced differences in character of growth were evident.

From the external appearance of the tops, it is apparent that tomato plants can be grown in a solution including both  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  over a relatively wide range of pH values without significant differences. But, as will be shown later, the rates of absorption of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  by these plants differed remarkably over the pH range used. That is, the external appearance of the plants was quite deceptive, considered from the standpoint of the activity of the plants with respect to nitrogen absorption.

Only the roots showed visible differences in character as a result of the different pH treatments. The roots of plants grown in the pH 4 and pH 5 solutions were very white and had many short laterals, but the roots of plants grown in the pH 6 and pH 7 solutions were not quite so white and had fewer short laterals. The roots in the pH 6 and pH 7 solutions were longer and more abundant, however, than the roots in the pH 4 and pH 5 solutions. The total root systems grown in the pH 6 solutions were the largest.

*Nitrogen absorption.*—The absorption rates in terms of milligrams per 100 gm. of green tissue in 6 hours, as listed in table 3, are represented diagrammatically in figure 1. Here, the bars on the left of the vertical zero line represent the average values of nitrogen absorbed as the anion ( $\text{NO}_3^-$ ) in milligrams per 100 gm. of green plant tissue during the absorption interval of 6 hours, and

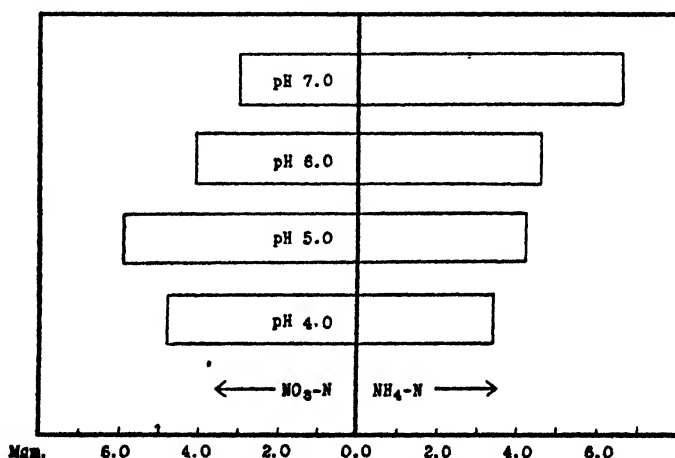


FIG. 1. MILLIGRAMS OF NITROGEN ABSORBED PER 100 GM. GREEN TISSUE IN 6 HOURS BY PLANTS 41 DAYS OLD

those on the right represent the corresponding cation ( $\text{NH}_4^+$ ) absorption values.

Figure 1 brings out clearly the pronounced influence of the pH of the culture solution on the rates of absorption of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  when these two forms are present simultaneously in the culture solution in approximately equal proportions.

It is evident that nitrogen in the cation form ( $\text{NH}_4^+$ ) was absorbed much more readily when the pH of the culture solution was relatively high (pH 7) than it was when the pH of the solution was relatively low (pH 4). The rate of absorption of the cation at pH 7 was nearly double that at pH 4. Conversely, nitrogen in the anion form ( $\text{NO}_3^-$ ) was absorbed much more readily at a relatively low pH (pH 4 to pH 5) of the culture solution than it was at the high pH 7. The rate of absorption of the anion at pH 7 was about one-half that at pH 5. The apparent exception to the general trend, indicated by the lower

rate of  $\text{NO}_3\text{-N}$  absorption at pH 4 than at pH 5, may have been due to a slightly unfavorable influence of the pH 4 solution on some phase of the metabolism of the plants, an influence which, in turn, might have affected the rate of absorption of  $\text{NO}_3\text{-N}$ . A suggestion of such an influence was observed in connection with the analytical results, which showed that the soluble organic nitrogen in the roots of the plants grown in the pH 4 solution was noticeably low in comparison with that present in the roots of plants grown in the pH 5, pH 6, and pH 7 solutions.

Furthermore, it is evident from figure 1, that at pH 4 the rate of absorption of  $\text{NH}_4\text{-N}$  was lower than that of  $\text{NO}_3\text{-N}$ , whereas at pH 7 the rate of absorption of  $\text{NH}_4\text{-N}$  was much higher than that of  $\text{NO}_3\text{-N}$ . It should be pointed out here that the data of table 2 in comparison with those of table 3 give evidence of the relationship between the relative rates of absorption of nitrogen as cations and anions and the changes in pH of the culture solutions. It is recognized that data are available for only two of the ions in the culture solution, but it is interesting to note that when the rate of removal of  $\text{NO}_3\text{-N}$ , the anion, from the solution clearly exceeded the rate of removal of  $\text{NH}_4\text{-N}$ , the cation, the pH of the solution increased; whereas, when the rate of removal of the cation distinctly exceeded that of the anion, the pH of the solution decreased. Also when nearly equal absorption rates prevailed, the pH of the solution remained nearly the same during the absorption test interval. Theron (43) has listed several ways in which this relationship could be interpreted.

#### *Results with plants 52 days old*

*Experimental procedure.*—The second absorption test was made 11 days after the first test, with plants grown at the same time and treated in exactly the same manner as were those of the preceding series harvested after the first absorption test had been completed. Solutions at two pH values only, pH 4 and pH 7, were here employed. In addition, several transfers were made 12 days before this absorption test was begun. Triplicate cultures previously grown in the pH 7 solution were transferred to the pH 4 solution and grown in the latter for the remainder of the experimental period. Likewise, triplicate cultures previously grown in the pH 4 solution were transferred to the pH 7 solution and grown in it to the end of the experimental period. The remainder of the cultures of this series grown in both the pH 4 and pH 7 solutions were not transferred, so that a comparison could be made between cultures grown continuously for 25 days in the pH 4 and pH 7 solutions, and cultures grown in these solutions for the first 13 days and then transferred to solutions of opposite reaction for the final 12 days. In every case, the absorption by the plants was tested in a solution of the same pH as that in which they had been grown for the final 12 days.

The plants used for this second absorption test had grown considerably during the 12 days after the transfers were made. Accordingly, 5 days before the second absorption test was begun, the rate of continuous renewal of the

culture solutions had been increased to 2 liters in 14 hours. Even this rate of solution renewal was not great enough to prevent a slightly greater change in pH of the solutions than that caused by the younger plants of the preceding series. This fact is brought out by table 4, in which the values presented were obtained in exactly the same manner as were those given in table 2. It will be observed that the plants 52 days old caused a change in pH of the solutions which was of the same order, although differing slightly in degree, as that caused by the plants 41 days old.

Certain modifications in technique were necessary in testing the absorption rates of the larger plants of this series. Two liters of test solution was used for each culture during the absorption interval instead of the 1 liter used with

TABLE 4  
*Changes in pH of the culture solutions brought about by plants 52 days old*

ORIGINAL pH OF SOLUTION IN RESERVOIRS	pH OF SOLUTIONS FROM OUTLETS OF CULTURE VESSELS BEFORE ABSORPTION TEST	FINAL pH OF SOLUTIONS USED FOR ABSORPTION TEST
4.0	4.0-4.2	4.96-5.5
7.0	5.4-5.8	6.1-6.3
7.0	5.2-5.75	6.0-6.15
4.0	4.1-4.2	5.2-5.6

TABLE 5  
*Rates of absorption of  $NH_4-N$  and  $NO_3-N$  by plants 52 days old*

pH OF CULTURE SOLUTIONS		AVERAGE GREEN WEIGHT PER PLANT	N ABSORBED PER 100 GM. GREEN TISSUE IN 6 HOURS		
Original	Final 12 days and test		$NH_4-N$	$NO_3-N$	Total
		gm.	mgm.	mgm.	mgm.
4.0	4.0	139.0	2.2	7.6	9.8
4.0	7.0	140.9	5.1	5.2	10.3
7.0	7.0	117.8	5.0	8.4	13.4
7.0	4.0	126.3	2.4	5.7	8.1

the preceding series. Of this, 850 cc. was placed in each of the quart jars used as culture vessels in this test. The remainder was dripped through the culture vessel during the absorption interval of 6 hours, so that the rate of renewal was approximately the same as that prevailing before the test. This was about twice the rate of renewal employed with the cultures of the preceding series during the absorption interval.

Each datum in table 5 is the average for triplicate cultures including three plants each. The same units and method of calculation were employed as for the data of table 3. Although the data in table 5 are based on the absorption from twice the amount of solution used per culture for the test of the preceding series, the solution volume per unit of plant tissue was approximately the same in both cases.

*Appearance of plants.*—Table 5 indicates differences in the average green weights per plant, but the differences probably should not be interpreted as resulting from the pH treatments received during the final 12 days of the experimental period. Instead, they might be traced to the slightly greater size of the plants grown in the pH 4 solution during the early part of the experimental period. However, the plants were all vigorous, and those of each culture in the series had from one to six open blossoms. The roots were healthy and growing, although they were not so white as those of the younger plants of the preceding series. In particular, a light brown tinge was evident on those grown in the pH 7 solution from the beginning of the experimental period.

*Nitrogen absorption.*—The absorption rates listed in table 5 are represented diagrammatically in figure 2, in which the same method of expressing results

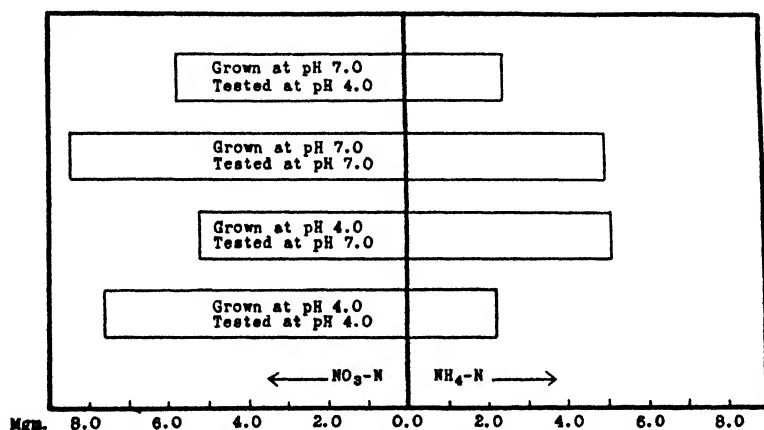


FIG. 2. MILLIGRAMS OF NITROGEN ABSORBED PER 100 GM. GREEN TISSUE IN 6 HOURS BY PLANTS 52 DAYS OLD

Transfers as indicated in the diagram were made 12 days before the absorption tests were begun.

is employed as in figure 1, with the addition of notes indicating the previous history of the plants. The results shown in table 5 and figure 2 will first be examined alone and then compared with those in table 3 and figure 1.

In the first place, the influence of the pH of the solution on the rate of absorption of  $\text{NH}_4\text{-N}$ , the cation, by the plants 52 days old is quite evident. The pH of the culture solution in which the plants were grown during the final 12 days of the experimental period determined the rate of absorption of  $\text{NH}_4\text{-N}$ , as is clearly indicated in figure 2. Those plants grown during the final 12 days and tested in the pH 7 solutions absorbed about twice as much  $\text{NH}_4\text{-N}$  as those grown during the same period and tested in the pH 4 solutions. Thus, it is clear that high rates of absorption of  $\text{NH}_4\text{-N}$  correspond to solutions of high pH values, and low rates of absorption of  $\text{NH}_4\text{-N}$  correspond to solutions of low pH values. This relationship is quite definite.



On the other hand, the average absorption rates of  $\text{NO}_3\text{-N}$  indicated for plants grown continuously in solutions at pH 7 differed only slightly from those indicated for plants grown continuously at pH 4, the advantage being in favor of those grown at pH 7. The absorption rates of  $\text{NO}_3\text{-N}$  were relatively high for these two sets of cultures. The data indicate that when the plants were at this stage of development the pH value of the culture medium, within the limit employed, did not exercise the same dominating influence in determining the rates of  $\text{NO}_3\text{-N}$  absorption as it did in determining the rates of  $\text{NH}_4\text{-N}$  absorption.

The plants which were transferred from solutions at pH 7 to those at pH 4, as well as those which were transferred from solutions at pH 4 to solutions at pH 7, 12 days prior to the time when the absorption tests were made, showed much lower average rates of  $\text{NO}_3\text{-N}$  absorption than did plants grown continuously in solutions having the same pH values as those in which the tests were made. It is apparent that the effect of transferring the plants from solutions of one pH to those of another was to retard the rates of  $\text{NO}_3\text{-N}$  absorption, regardless of whether the transfers were made from solutions of relatively high to those of low pH values, or vice versa. Furthermore, this retarding influence appears to have been effective over a considerable period of time, in the present instance, 12 days from the time when the transfers were made.

The pH of the solution thus seems to have been the dominating factor in determining the rate of absorption of nitrogen as the cation ( $\text{NH}_4^+$ ), but it was not the main factor in determining the rate of absorption of nitrogen as the anion ( $\text{NO}_3^-$ ) when the plants were at this stage of development.

#### *Results with plants 38 days old*

Further evidence in support of the foregoing conclusions is presented in table 6 and in figure 3, which include the results obtained with a supplementary series of cultures, in which the plants of one set of cultures were transferred from solutions at pH 7 in which they had been grown continuously to solutions at pH 4 for immediate tests of the absorption rates. Also, another set of cultures was transferred from solutions at pH 4 in which they had been grown continuously to solutions at pH 7 for immediate tests of the absorption rates. The main purpose of the tests was to determine whether change in absorption rates with alteration in the pH values of the culture solutions, as indicated for the plants in the preceding series, is an immediate effect or whether the rate change due to the change in pH of the culture solution is a gradual process of adjustment taking place over a relatively long interval of time. These particular tests were made with plants 38 days old, grown in the same manner as were those of the two series already described, but grown in midsummer, whereas those of the two preceding series were grown during the spring months. The results are quite comparable, although a different unit of plant tissue is used as a basis for expressing the absorption rates, a change made necessary by the lack of data on the green weight of the roots.

As is indicated by the data of table 6, represented diagrammatically in figure 3, the immediate and decisive effect of the pH value of the culture solution on the rates of absorption of  $\text{NH}_4\text{-N}$  is unquestionable. Plants grown in the pH 4 solutions and transferred to and tested in the pH 7 solutions absorbed four times as much  $\text{NH}_4\text{-N}$  during the 6-hour test interval as did those grown and tested in the pH 4 solution. In fact, the average rate of absorption of  $\text{NH}_4\text{-N}$  by the plants so transferred closely approximated that of plants grown and tested in the pH 7 solution. On the other hand, the plants grown in the solution at pH 7 and then transferred to and tested in the pH 4 solution absorbed less than half as much  $\text{NH}_4\text{-N}$  as did those grown and tested in the pH 7 solution. However, the average rate of the absorption of  $\text{NH}_4\text{-N}$  by the plants thus transferred was still considerably greater than that of plants grown and tested in the pH 4 solutions. Here, again, high average rates of absorption of  $\text{NH}_4\text{-N}$  correspond

TABLE 6

*Rates of absorption of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  by plants 38 days old. Transfers made at the time of the absorption test*

pH OF SOLUTION		N ABSORBED PER GM. DRIED TISSUE IN 6 HOURS		
Before test	During test	$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$	Total
		mgm.	mgm.	mgm.
4	4	0.25	1.13	1.38
4	7	0.99	0.70	1.69
7	4	0.46	0.81	1.27
7	7	1.09	0.62	1.71

to relatively high pH values of the culture medium, and low rates correspond to low pH values of the medium.

When studying the rates of absorption of  $\text{NO}_3\text{-N}$  by those cultures in which no transfers were made just prior to the time of making the absorption tests, it is found that the opposite relation holds between pH of the medium and average rates of  $\text{NO}_3\text{-N}$  absorption. That is, high average rates of  $\text{NO}_3\text{-N}$  absorption correspond to low pH values of the culture solution and low rates correspond to high pH values, although this relation is not always so clearly defined as is the opposite relation between pH of the culture solution and  $\text{NH}_4\text{-N}$  absorption. With these plants, the disturbing influence of pronounced reaction change of the culture medium upon the  $\text{NO}_3\text{-N}$  absorption rates is very apparent. This is clearly brought out by the fact that the plants grown in solutions at pH 7, then transferred to and tested in solutions at pH 4, showed much lower  $\text{NO}_3\text{-N}$  absorption rates than did the plants grown and tested in solutions at pH 4.

If now, the  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  absorption rates of the plants transferred from the pH 4 to the pH 7 solutions, as shown in figure 3, are compared with the corresponding rates of the plants transferred from the pH 7 to the pH 4

solutions, it is clear that the great difference between the average  $\text{NH}_4\text{-N}$  absorption rates for these two sets of cultures emphasizes the immediate and determinative effect of the pH of the solution on the rate of absorption of the cation. Although the immediate and determinative influence of pH of the culture solution on the  $\text{NO}_3\text{-N}$  absorption rates is also apparent, the effect is not so pronounced nor so clearly defined as it is in the case of the cation absorption rates.

*Influence of age of plants upon absorption*

If the data of the older plants (table 5 and figure 2) are considered again in comparison with the data of the younger plants (table 3 and figure 1) the following points of agreement may be observed: (a) the average absorption rates of

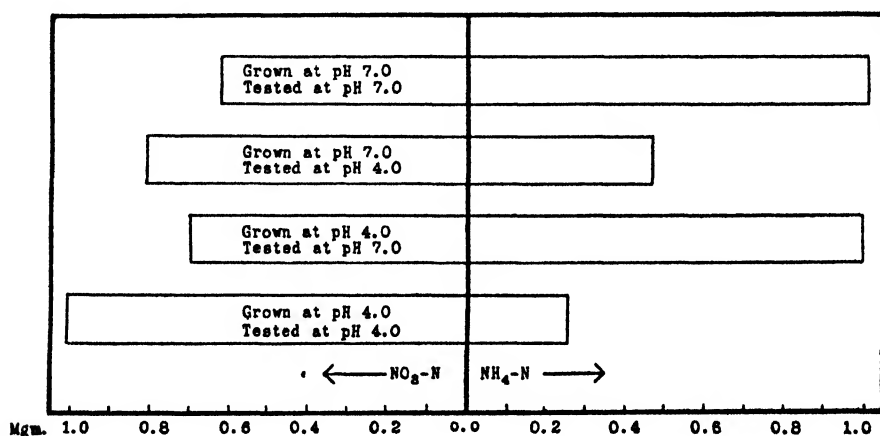


FIG. 3. MILLIGRAMS OF NITROGEN ABSORBED PER GRAM DRY PLANT TISSUE IN 6 HOURS BY PLANTS 38 DAYS OLD

Absorption tests were begun immediately after the transfers, as indicated in the diagram, had been made.

$\text{NH}_4\text{-N}$  were greater at high than at low pH values of the culture solutions, and (b), the average rates of absorption of  $\text{NH}_4\text{-N}$  were always lower than were those of  $\text{NO}_3\text{-N}$  from the pH 4 solutions. These points of agreement are due largely to the determinative effect of the pH of the culture solutions on the rates of absorption of  $\text{NH}_4\text{-N}$ .

A further comparison of these data brings out the following points of apparent disagreement: (a) The rate of absorption of  $\text{NO}_3\text{-N}$  by the older plants was not greatly influenced by the pH of the solution, which is in direct contrast to the results obtained with the younger plants; and (b) the rates of absorption of  $\text{NO}_3\text{-N}$  from the pH 7 solutions by the older plants were always higher than the rates of absorption of  $\text{NH}_4\text{-N}$  from the corresponding solutions, whereas with the younger plants the rates of absorption of  $\text{NH}_4\text{-N}$  from pH 7 solutions always greatly exceeded those of  $\text{NO}_3\text{-N}$ . The cause of these two differences

between the older and younger plants must be sought in the influence of the age of the plants upon the rates of absorption of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ .

The results here reported on the effect of the age of the plant on the absorption rates of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  are in excellent agreement with those of earlier studies by Jones and Skinner (17); Maftel (27); Prince, Jones, and Shive (35); and Stahl and Shive (41, 42). For example, Stahl and Shive, using a similar technique with buckwheat, found that the rate of absorption of  $\text{NH}_4\text{-N}$  per unit of plant tissue decreased as the plants grew older, whereas the rate of absorption of  $\text{NO}_3\text{-N}$  per unit of plant tissue increased until the flowering stage was reached. Similarly, with the tomato plants here investigated, the data for those grown in both the pH 4 and the pH 7 solutions show that the rates of absorption of  $\text{NH}_4\text{-N}$  decreased as the plants became older and the rates of absorption of  $\text{NO}_3\text{-N}$  increased. A full explanation of these facts is not yet possible.

#### THEORETICAL CONSIDERATIONS

In these experiments, no attempt was made to study the rates of absorption of any other ions than the  $\text{NH}_4\text{-ion}$  and the  $\text{NO}_3\text{-ion}$  from the culture solution, nor was any study made of the possible excretion of ions or molecules by the plant. The results, therefore, are not complete enough in themselves to furnish the basis for any theory concerning the absorption and assimilation of elements by plants. Nevertheless, the pronounced influence of the pH of the culture medium upon the rates of absorption of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  leads to a consideration of the relationships of the results here obtained to theories which have been proposed relative to ion absorption.

In the results presented, the absorption of  $\text{NH}_4\text{-N}$  and of  $\text{NO}_3\text{-N}$  has been referred to as an absorption of ions. Yet it is recognized that the absorption of molecules by plant cells can not be excluded from consideration. Thus Osterhout (29) was led to believe from his work with *Valonia* that either it is principally molecules that penetrate the protoplasm, or that concentration (and perhaps reaction) in the protoplasm plays a more important rôle than diffusion constants. He does not imply that ionic penetration does not occur, but only that it must play a subordinate rôle because of the non-aqueous character of the protoplasmic surfaces.

Brooks (6) states that no non-aqueous solvents have been shown to be more permeable to KOH than to NaOH, as is apparently demanded by Osterhout's molecular hypothesis. Brooks (5) has advanced a hypothesis of ionic exchange. He suggests that the cell is surrounded by a plasma membrane consisting of a mosaic of anion-permeable and cation-permeable areas which are of the nature of charged porous films, that the diameters of the pores are variable according to experimental conditions, and that the penetrabilities of different ions are characteristic functions of their own effective diameters and of the diameters of the pores in the membranes. But he has admitted (6) that

no test permitting experimental discrimination between his hypothesis and that of Osterhout has yet been applied.

Osterhout (29) has criticized Brook's hypothesis for its assumption that anions could not accompany the H ion out of the cell and the subsequent use of the anions to calculate the passage of the Cl ion into the cell. Briggs (2) has given a mathematical treatment of the theoretical case of a membrane having certain areas permeable to anions and certain areas permeable to cations. This calculation casts doubt on the hypothesis of Brooks (5). Briggs (3) has also criticized Osterhout's mathematical formulation of the thermodynamic potential for molecular penetration by pointing out that although the formula would account for the accumulation of K within the cells, no mechanism is suggested for forcing HCl from the outside to the inside of *Valonia* cells against a thermodynamic potential.

Briggs and Petrie (4) and Briggs (2, 3), in turn, have offered several related but different hypotheses based on experiments on the absorption of salts by storage tissue, such as that from potato tubers. This is a special case of absorption, however, and since they did not study the same type of process as that reported in this paper, no comparisons will be attempted.

Butkewitsch (7) studied the passage of salts through collodion membranes and found that if the H-ion concentration of the solution containing the salts was increased, the diffusion of anions through the membrane was increased, or if the OH-ion concentration was increased, the diffusion of cations through the membrane was correspondingly increased. He also conducted other experiments with salts including different combinations of anions and cations, and concluded that molecules diffused through the membrane.

On the other hand, Hager and Stollenwerk (10) quoted an experiment by Michaelis and Fujeta with collodion membranes. Because HCl would not pass through the membrane until a KCl solution was substituted for water on the opposite side of the membrane, they concluded that the H ions could not pass the membranes until K ions were present to provide for an ionic exchange.

Mevius (24, 25) has interpreted his results with maize plants grown in solutions of different pH values as indicating that it is the  $\text{NH}_3$  or  $\text{NH}_4\text{OH}$  molecule which penetrates the cell and causes toxicity when the " $\text{NH}_3$ -tension" is too great on the outside. He pointed out (25), correctly, that the concentration of undissociated  $\text{NH}_4\text{OH}$  increases with increasing pH of the solution or with increasing concentration of an ammonium salt in a solution. Evidences of the rapid penetration of  $\text{NH}_3$  or  $\text{NH}_4\text{OH}$  into tissues have been presented by Harvey (11).

Hager and Stollenwerk (10) supported the view of Mevius (23) regarding the penetration of  $\text{NH}_4\text{OH}$  into cells, and elaborated upon his hypothesis by noting the effect of the hydration of the anion of the ammonium salt upon the penetration of ammonia into the plant. They suggested the term "membrane hydrolysis" a hydrolytic cleavage of the salts which have highly hydrated anions and a subsequent absorption of free ammonia without the anion. However,

in their discussion of the absorption of various forms of ions from solutions, Hager and Stollenwerk did not exclude the possibility of ion-exchange, especially with the H ions and  $\text{HCO}_3$  ions of the cell.

Pantanelli (31), after many experiments on the absorption of ions by plants, concluded that normal, living, uninjured protoplasm absorbs individual ions by adsorption, saying that the assumption of a separate absorption of free acids and bases is superfluous, since the natural electric charge of the plasma colloid produces an adsorption potential leading to an ionic separation. He has admitted, however, that it is well known that an entire series of non-dissociated or weakly dissociated organic substances are taken up by the cell in molecular form.

Jenny and Cowan (16) studied the removal by plants of Ca ions adsorbed on purified colloids and concluded that the plant obtained the Ca ions by an excretion of H ions; that is, that an ionic exchange between plant and colloid medium took place.

It is evident that there are supporters of both conceptions, of ionic or molecular absorption of elements by plants, and that there is much to be said in support of both sides of the question.

It is interesting, in this connection, to speculate with reference to how the influence of the pH of the culture solutions here employed on the rates of absorption of  $\text{NH}_4\text{-N}$  and of  $\text{NO}_3\text{-N}$  could be interpreted according to the various theories mentioned. According to Osterhout's (29) theory of molecular absorption, the thermodynamic potential, represented by the product of the activities of the ions ( $\text{NH}_4^+$ ) ( $\text{OH}^-$ ), would increase on the outside of the plant as the OH-ion concentration increased. This would tend to force more  $\text{NH}_4\text{OH}$  into the cell from a solution of high pH than from one of low pH. This is in direct accord with observation in these experiments. Conversely, a similar method of reasoning would lead to the conclusion that the thermodynamic potential ( $\text{H}^+$ ) ( $\text{NO}_3^-$ ) would be greater at a low pH than at a high pH, so that  $\text{HNO}_3$  would be absorbed more rapidly from a solution of low pH than from a solution of high pH. This is also in accord with experimental evidence obtained with the younger plants in the present work.

The theory of Mevius (25), that a greater " $\text{NH}_3$ -tension" would result from the greater hydrolysis of an ammonium salt at a high pH than would occur at a low pH, and hence, that a higher rate of absorption of  $\text{NH}_3$  would take place from a solution of high pH than of low pH, is likewise supported by the experimental data here provided.

Qualitatively, these data appear to be consistent with the theories outlined. Yet it should be pointed out that an increase of only 2 pH units in the culture solution would increase the thermodynamic potential suggested by Osterhout times, or would increase the concentration of un-ionized,  $\text{NH}_4\text{OH}$  emphasized by Mevius nearly 100 times. Yet the rate of absorption of  $\text{NH}_4\text{-N}$  from the pH 7 solutions was never more than four times that from the pH 4 solutions. It would appear, therefore, that the rates of absorption of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$

are conditioned by other factors than the thermodynamic potential suggested by Osterhout, or the molecular concentration suggested by Mevius.

It is not possible to correlate in any satisfactory manner the theories of Brooks (5) or of Briggs and Petrie (4) and Briggs (2, 3) with the data here presented.

Attempts have been made to apply the Donnan membrane equilibrium to the absorption of ions by plants. For example, Osterhout (29) has shown that an attempt to extend the Donnan principle to the distribution of electrolytes within and without the cells of *Valonia* is unsuccessful.

Briggs and Petrie (4) also found that the conception of a simple Donnan membrane equilibrium operating between two homogeneous phases is inadequate to explain the phenomena of ionic intake by plants. They extended the scope of the Donnan principle by postulating a greater number of phases in the tissue.

Mattson (21) has applied the laws governing the Donnan equilibrium to the case of a colloidal micelle where an actual membrane does not exist. The success of this application suggests that Donnan equilibria might exist around colloidal micelles in the protoplast. However, the possible existence of more than one equilibrium and the presence of many ions in the solution make it impossible to apply the Donnan equilibrium to the data presented above.

Pantaneli's (31) theory of absorption emphasizes the colloidal properties of the protoplast, and regards absorption as an adsorption process. It appears that any discussion of the absorption or assimilation of elements ought not to neglect the colloidal nature of the protoplast. Heilbrun (12) states that the charge on the outer surface of a cell would be largely determined by ions present in the external medium. Briggs (3) quotes Stiles as suggesting that the relation between the amount of ions absorbed per unit volume of tissue and the final concentration is expressed by the Freundlich isotherm for absorption and that, hence, absorption can not be a simple process of diffusion through a membrane.

In support of these ideas, Robbins (37) found that potato tuber tissue behaves like an ampholyte with an isoelectric point near pH 6.4, basic dyes being absorbed and more strongly retained above pH 6, and acid dyes being absorbed and more strongly retained below pH 6. However, he noted that the absorption of either class of dyes did not stop at the isoelectric point, but continued over to the other side of the possible isoelectric point.

Tiedjens<sup>3</sup> has extended Raber's (36) theory of permeability, which is based on the charge on the cell membrane, to apply to the protoplast as a whole. Considering the protoplast as a whole, Tiedjens pointed out the possible effect of the pH of the culture solution on the dispersion of protein complexes and on the dissociation of soluble ampholytes in the protoplast, and correlated these possi-

<sup>3</sup> Tiedjens, V. A. Factors affecting assimilation of ammonium and nitrate nitrogen, particularly in the tomato (*Lycopersicum esculentum*, Mill.) and in the apple (*Pyrus malus*, L.). Unpublished data.

ble effects with the data he obtained from tomatoes grown in solutions of different pH values. He found that  $\text{NH}_4\text{-N}$  was best assimilated when the pH value of the culture solution was above that of the plant sap, whereas  $\text{NO}_3\text{-N}$  was best assimilated when the pH value of the culture solution was below that of the composite plant sap. He suggested that those ampholytes of the cell which were in a medium above their isoelectric points would combine with cations, such as  $\text{NH}_4^+$ , whereas those ampholytes in a medium below their isoelectric points would combine with anions, such as  $\text{NO}_3^-$ . Hence, when the pH of the solution was high, combination with cations was promoted, and when the pH of the solution was low, combination with anions was favored.

This theory, it should be noted, implies that assimilation must take place near the surface of the protoplast which is in contact with the culture medium, unless the cell solution bathing the protein complexes or including the soluble ampholytes within the protoplast is also affected by the pH of the external culture solution. Theron (43) found that the pH of the juices from the tops of plants grown in solutions of different pH values was not affected by the pH of the culture solutions, but that the pH of the expressed sap from the roots was influenced by that of the culture solutions. When the pH range of the external solutions was from 3.5 to 8.5 he found that the maximum range in the juices from the roots was about 1 pH unit. Keysener (19) also found a difference in pH of the root juices of plants grown in culture solutions of different pH values. It is conceivable that the magnitude of difference in pH of the external solutions might be enough to influence the rates of absorption and assimilation of  $\text{NH}_4\text{-N}$  and of  $\text{NO}_3\text{-N}$  as suggested by Tiedjens. The data reported in this paper are in harmony with the theory emphasized by Tiedjens.

Mention should also be made of the work of Niklewski, Krouse, and Lemanczyk (28). They interpreted their results by asserting that the permeability of the plasma membranes depends upon various fluctuations in the degree of dispersion of the plasma, which is influenced by the mineral constituents taken up by the cells. They considered the root colloids to be negatively charged above pH 4 in solutions of  $\text{Ca}(\text{NO}_3)_2$  in order to explain the greater absorption of  $\text{Ca}^+$  than of  $\text{NO}_3^-$  from solutions above pH 4.

Unfortunately, a quantitative comparison of the aforementioned colloidal theories of absorption and assimilation with the data obtained in the experiments here considered can not be made, since the theories are not stated in quantitative form.

It is not at all surprising that no simple and complete picture of the mechanism of the influence of the pH of the culture solution on the rates of absorption and assimilation of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  can be presented. Each theory discussed emphasizes only relatively few of the total number of components of the system composed of the plant and its environment, showing how these few components might be affected by a change in the pH of the culture solution. But the plant and its environment make up a complex system with many components which may possibly be affected by a change in any one of them.



Thus changing the pH of the culture solution does affect the ionic and dissociation equilibria within that solution and may conceivably affect such things as the electrostatic charges on the surface of the protoplasm and on the interior of the protoplast, the degree of dispersion of the colloidal complexes of the protoplast, the dissociation of ampholytes within the protoplasm, the pH of the composite sap of the roots as a whole or of specific tissues therein, the buffer systems of the roots, and the  $\text{CO}_2$  or  $\text{HCO}_3^-$  equilibrium between the plant and the solution. Furthermore, a change in any one of these components may influence one or more of the other components in a secondary way. Indeed, on account of the complexity of the system, any attempt to interpret all of its activities based only on the behavior of a few of the active components of the whole system must of necessity be incomplete.

#### SUMMARY

Tomato plants were grown in continuously renewed and continuously aerated, complete culture solutions including approximately equal proportions of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ , the solutions being adjusted to pH 4.0, 5.0, 6.0, and 7.0. The rates of absorption of  $\text{NH}_4\text{-N}$  and of  $\text{NO}_3\text{-N}$  from the solutions by the plants were determined at two different ages. In addition, the plants were harvested at the time of each absorption test and analyses of the tissue for certain nitrogenous fractions were made. The following results were obtained:

##### *Absorption by plants 41 days old*

The rates of absorption of  $\text{NH}_4\text{-N}$  were higher from solutions of high pH than from solutions of low pH, the rates of absorption at pH 7 being nearly twice those at pH 4.

The rates of absorption of  $\text{NO}_3\text{-N}$  were higher from solutions of low pH than from solutions of high pH, the rates of absorption at pH 7 being about one-half those at pH 5, where the maximum rates occurred.

At pH 4 the  $\text{NH}_4\text{-N}$  absorption rates were lower than the  $\text{NO}_3\text{-N}$  rates. At pH 7 the  $\text{NH}_4\text{-N}$  absorption rates were higher than the  $\text{NO}_3\text{-N}$  rates.

The maximum rates of  $\text{NH}_4\text{-N}$  absorption were higher than the maximum rates of  $\text{NO}_3\text{-N}$  absorption in the younger plants.

##### *Absorption by plants 52 days old*

In agreement with the results obtained with the plants 41 days old, the rates of absorption of  $\text{NH}_4\text{-N}$  were higher at pH 7 than at pH 4.

The rates of absorption of  $\text{NH}_4\text{-N}$  were much lower than those of  $\text{NO}_3\text{-N}$  from the pH 4 solutions. These similarities were due to the determinative effect of the pH of the solutions on the rates of nitrogen absorption.

In contrast with the results obtained with the younger plants, the rates of absorption of  $\text{NO}_3\text{-N}$  in the older plants were not so greatly influenced by the pH of the solution as they were in the younger plants.

Within the range of pH values employed, the rates of absorption of  $\text{NO}_3\text{-N}$

were higher than those of  $\text{NH}_4\text{-N}$  absorption from solutions of corresponding pH. This emphasizes the influence of the age of the plants upon the relative rates of absorption of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ .

Within the period investigated, the rates of absorption of  $\text{NH}_4\text{-N}$  per unit of plant tissue decreased, and the rates of absorption of  $\text{NO}_3\text{-N}$  increased as the plants became older.

### *Effect of reaction change*

The effect of transferring plants from solutions of one pH to those of another pH, 12 days before the absorption test, was to retard the rates of absorption of  $\text{NO}_3\text{-N}$ , regardless of whether the transfers were made from solutions of relatively high to those of low pH values, or vice versa.

Plants transferred from pH 7 solutions to pH 4 solutions or vice versa for immediate absorption tests, showed that the effect of the pH on the rates of absorption of  $\text{NH}_4\text{-N}$  was immediate and decisive.

Although reaction change exerted an immediate and marked disturbing influence upon the rates of  $\text{NO}_3\text{-N}$  absorption, the effect was not so pronounced or so clearly defined as it was upon the rates of  $\text{NH}_4\text{-N}$  absorption.

Outstanding theories of absorption and permeability are discussed in relation to their bearing upon the results obtained in these investigations.

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# A STUDY OF RHIZOBIUM SPECIES IN RELATION TO NODULE FORMATION ON THE ROOTS OF FLORIDA LEGUMES: II<sup>1</sup>

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## NITROGEN-FIXATION STUDIES

The ability of the nodule bacteria of Leguminosae to fix nitrogen is a property of much concern from a practical point of view. It has been studied by several investigators in connection with cultures representing some of the more important cross-inoculation groups. Stevens (17), working with Rhizobia from alfalfa and sweet clover, found certain "sub-strains" to be much more efficient than others in this respect. The power of nodule production was about the same, since the low nitrogen-fixers formed as many nodules per plant as did the ones possessing power of greater nitrogen assimilation. Similar results have been reported by Wright (23) for different cultures of soybean bacteria; by Sears and Clark (14) and by Hansen and Tanner (6) with navy and valentine beans; and by Whiting, Fred, and Helz (22) with cultures isolated from the field pea. In the latter case, inoculation with the more efficient nitrogen fixers raised the percentage of nitrogen, increased the yield, and raised the proportion of fancy table peas (sizes 2 and 3) to low grade peas (sizes 4 and 5).

In view of the aforementioned reports, it seemed desirable to conduct nitrogen-fixing experiments with the cowpea and soybean with certain cultures of their nodule-producing bacteria.

The tests were made with sand cultures in 1-gallon pots. The procedure and technique employed were the same as those described in section I for cross-inoculation work.<sup>3</sup> The experiment for each culture was run in replicates of three pots with three plants each. The plant nutrient administered was Bryan's modification of Crone's nitrogen-free solution, the formula for which has been given elsewhere. The experiments were observed daily, the moisture content corrected to approximately 60 per cent, saturation twice a week, and the nutrient solution given once a week. The duration of the tests was 11 weeks, or up to the fourth or fifth day after the first blooms appeared.

Figure 1 of plate 1 shows a representative one of each replicate of the three pots from seven of the nine tests at this stage of development. In examining

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<sup>2</sup> The serological work was done in cooperation with Miss Hellen Jeffery (Kentucky Board of Health).

<sup>3</sup> *Soil Sci.* 37 (2), February 1934.

the photograph, it should be borne in mind that all the conditions were constant except that of inoculation, which varied. The plants are arranged in order of

TABLE 1  
*Efficiency of cowpea and soybean bacteria on cowpea plants*

CULTURE USED	REPLICATE NO.	OVEN-DRY WEIGHT OF 3 PLANTS	NITROGEN	NITROGEN IN PLANTS	GAIN IN NITROGEN*
		gm.	per cent	mgm.	mgm.
Controls.....	1	1.86	1.260	23.4	.....
	2	1.66	1.350	22.4	.....
	3	2.22	1.370	30.4	.....
Soybean 6.....	1	5.10	3.480	177.5	152.1
	2	6.19	2.650	164.0	138.8
	3	5.48	3.105	170.1	144.7
Cowpea 11.....	1	6.49	3.125	202.8	177.4
	2	7.63	3.390	258.6	233.2
	3	6.89	3.685	253.9	228.5
Soybean 915.....	1	6.87	3.075	211.3	185.9
	2	7.86	3.480	273.5	248.1
	3	8.29	2.975	246.6	221.2
Soybean 1.....	1	6.80	3.970	269.9	244.5
	2	6.71	3.035	203.6	178.2
	3	7.22	3.470	250.5	225.1
Soybean 13.....	1	8.35	3.835	320.2	294.8
	2	7.99	3.980	318.0	292.6
	3	8.00	3.980	318.4	293.0
Cowpea 10.....	1	9.15	4.580	419.1	393.7
	2	8.22	4.455	366.2	340.8
	3	8.42	4.370	367.9	342.5
Cowpea 2.....	1	7.35	4.940	363.0	337.6
	2	8.33	4.615	384.4	359.0
	3	8.93	4.010	358.0	332.6
Cowpea 28.....	1	8.21	3.675	301.7	276.3
	2	8.90	3.740	332.8	307.4
	3	8.60	3.790	325.9	300.5

\* Difference between the average amount of nitrogen in the three control plants (25.4 mgm.) and the amount of nitrogen found in the inoculated plant.

a graduation from the poorest to the best with regard to efficiency of the cultures as nitrogen fixers. The plants in pot 1 (the control) attained most of

their growth during the first 6 weeks. They were yellow and sickly in appearance. Although the photograph does not show it very well, there was a gradation in intensity of color from left to right, the color range reaching a dark green in pots 6 and 7. The results indicate clearly a variation in the nitrogen-fixing power of the *Rhizobia* from the nodules of cowpeas and soybeans when working in symbiosis with the cowpea plant.

After the photographs were taken, the plants were harvested; the tops being cut at the surface of the sand. The roots were then washed free of sand, and the nodules per plant counted. The number of nodules ranged from 63 to 105 per plant, without respect to cultures. After being dried over night at 100°C. or slightly above, the plants were weighed, and their nitrogen content was determined by the "Gunning Method." The data from these determinations are given in table 1 and confirm the idea conveyed by the photograph.

Nitrogen-fixing tests similar to these described were conducted with some of the aforementioned cultures and the soybean host plant. Cowpea cultures 2 and 10 had to be omitted, however, since they do not produce nodules on the roots of the soybean plant (just one of the unexplained but common inconsistencies exhibited by the legume nodule bacteria of the cowpea cross-inoculation group). No analyses were run on the soybeans, but every indication is that the results would have been similar to those obtained with cowpeas.

Figure 2 of plate 1 shows these soybeans plants arranged with an uninoculated control at the left, the poorest appearing inoculated plants next in order and on through with the better ones of the group at the right.

The experiment with the cowpeas described in the foregoing was repeated in the greenhouse with a sort of supplementary field test. The greenhouse tests were conducted in duplicate with only five cultures. The field supplement was not conducted entirely in keeping with the custom for such tests. In this case, the extra plants at the time for thinning (6 weeks) in the greenhouse test, were removed from the pots with sterilized forceps and spatulas to small plats prepared in the field. The usual procedure for field tests is to plant the seeds in soil.

All of the inoculated plants showed an abundance of nodules, without respect to cultures used, at the time of transplanting. Two of the cultures used had begun to show signs of superiority (greater plant development) at this time. These continued to develop while the other inoculated plants, as well as the controls, showed a considerable lag period. After approximately 3 weeks, however, these, including the uninoculated controls, darkened in color and developed rather rapidly, as a result, no doubt of inoculation in the soil, until the end of the twelfth week, when all of them were photographed and harvested.

The greenhouse portion of the foregoing experiment was not entirely satisfactory. During the last 3 weeks of the test, the temperature under glass was too high for the best development of the cowpea plants. In spite of this retarded growth, however, the plants show relative proportions and are included in the photograph taken of the field plats (pl. 1, fig. 3).



The uninoculated controls appear at the right of the photograph and are numbered 1. They are not very clear, because of poor light. In each case one of the duplicate pots from which the field plants were taken is elevated behind its respective field plat. The photograph with its legends gives the details concerning cultures used.

Figure 1 of plate 2 showing small plat 5, plants inoculated with cowpea culture no. 31, gives a fair idea of good inoculation under field conditions. The soil adjacent to the plat was removed to a depth of  $3\frac{1}{2}$  feet, then the roots were exposed by washing gently with water from a hose.

The foregoing data indicate that only certified nitrogen-fixing *Rhizobia* should be used in commercial culture work.

#### SEROLOGICAL PROPERTIES OF RHIZOBIA

In 1912, Zipfel (24) found that the antisera for some cultures of the legume nodule bacteria would not agglutinate certain others. Later (in 1914) Klimmer and Kruger (7), by agglutination and complement-fixation methods, pointed out a correlation between serological groups and Simon's (15) cross-inoculation group. The results of these investigations were confirmed in 1921 by Vogel and Zipfel (19). To this point, data concerning serological and cross-inoculation relationships seemed to be consistent and to coincide with each other.

However, Stevens (16) in 1923, working with 55 cultures representing 7 species of legume bacteria, found that *Rhizobia* belonging to any cross-inoculation group could be further separated into two or more sub-groups by the agglutination reaction. This investigator (17), in 1925, found differences also in the ability of the subgroups to fix nitrogen in symbiosis with their interchangeable host plants.

Bialosuknia and Klott (3) reported also, in 1923, that the same kind of plant from different sources had nodule bacteria with different agglutination properties. These investigators reported two cultures from the same nodule that differed in this respect.

Dunham and Baldwin (4) isolated two serologically different cultures of *Rhizobia* from the same leguminous plant, but failed to find more than one type of organism in the same nodule.

Wright (23), Walker (21), Hansen and Tanner (6), and others working along similar lines have confirmed these serological variations.

In considering the references given, it seems that the complement-fixation test was employed in the main for determining the serological relationship of legume nodule bacteria. Later both the complement-fixation and agglutination reactions were used. It seems, however, that in later years workers in this field have resorted more to the latter reaction for serological classification of these bacteria. The difficulty encountered in manipulating the technique of the complement-fixation test undoubtedly accounts for the unpopularity of this reaction. It was decided, however, to use both the tests in connection

with Rhizobia representing the different cross-inoculation groups under consideration here.

The technique followed in carrying out the complement-fixation studies is that described by Kolmer (8) for diagnosing bacterial diseases. In this method, the standardized antigen and complement are mixed with the immune serum in saline and placed in the ice box over night. The hemolytic system is

TABLE 2

*Results of complement-fixation tests with five antisera with antigens of various nodule bacteria*

ANTIGENS	ANTISERA				
	Bean 80	Clover 5	Cowpea 600	Lespedeza 6	Soybean 504
Alfalfa 2.....	—	—	0	—	—
Amorpha.....	—	—	0	—	—
Amphicarpa.....	+	++	0	++	—
Bean 17.....	++—	++	0	++	—
Bean 80.....	++++	—	0	—	—
Bean 405.....	++++	++	0	—	—
Black locust.....	—	—	0	—	—
Clover 4.....	—	++	—	—	—
Clover 5.....	Ac.	++++	±	—	—
Clover 45.....	—	±	—	—	—
Clover 200.....	—	—	—	—	—
Cowpea 600.....	—	—	++++	—	+
Crotalaria 81.....	±	—	++	—	—
Dalea.....	—	—	—	—	+
Lespedeza 6.....	—	±	+	++++	+
Lotus.....	++	—	±	—	—
Lupine B.....	—	—	—	—	+++
Lupine 16.....	—	—	—	—	+++
Lupine 801.....	—	—	—	—	—
Lupine 1.....	—	—	±	—	—
Pea 1.....	—	—	—	—	—
Sanfoin.....	—	—	—	—	—
Soybean 5.....	—	—	—	—	—
Soybean 68.....	—	—	±	—	+++
Soybean 504.....	—	—	—	—	++++
Vetch (kidney).....	—	—	—	—	—
Vetch (wild).....	—	—	—	—	—

Ac. = anticomplementary reaction of antiserum.

0 = no test, or unsatisfactory results.

then added, and the mixture incubated 1 hour at 37°C., at the end of which time the results are recorded.

The antisera used were from rabbits which had been immunized by injecting four doses of approximately 200,000,000 cells of nodule bacteria intraperitoneally at intervals of 6 days, and one half-size dose intravenously 5 days after the fourth intraperitoneal injection. The bacterial suspensions were prepared

by washing off several 5-day-old slant cultures and standardizing by the turbidity method of Gates (5). As the various cultures of *Rhizobia* differ greatly in opacity, this method was not very satisfactory. Seven days after the last injection the rabbits were bled from the heart. The blood was allowed to coagulate at room temperature. The firm clot was then released from the walls of the test tube with a small sterilized glass rod and the serum separated

TABLE 3

*Agglutination reaction of six antisera with various cultures of nodule bacteria*

ANTIGEN	ANTISERA					
	Bean 80	Clover 5	Cowpea 600	Lespedeza 6	Soybean 5	Soybean 504
Amorpha.....	—	—	—	—	—	—
Amphicarpa.....	—	—	—	—	—	—
Bean 80.....	1:2560	—	—	—	—	—
Bean 17.....	1:20	—	—	—	—	—
Bean 405.....	1:2560	—	—	—	—	—
Beggar-weed 400.....	—	—	1:2560	1:2560	—	—
Beggar-weed 402.....	—	—	1:2560	1:2560	—	—
Black locust.....	1:40	—	—	—	—	—
Clover 4.....	—	1:2560	—	—	—	—
Clover 5.....	—	1:2560	—	—	—	—
Clover 45.....	—	1:80	—	—	—	—
Cowpea 2.....	—	—	1:2560	1:2560	—	—
Cowpea 10.....	—	—	1:2560	1:2560	—	—
Cowpea 600.....	—	—	1:2560	1:2560	—	—
Crotalaria 81.....	—	—	—	—	—	—
Crotalaria 408.....	—	—	1:40	—	—	—
Crotalaria 409.....	—	—	—	—	—	—
Dalea.....	1:40	—	—	—	—	—
Kidney vetch.....	—	—	—	—	—	—
Lespedeza 6.....	—	—	1:1200	1:2560	—	—
<i>Lupinus vilosus</i> 1.....	—	—	—	—	—	—
Lupine 3.....	—	—	—	—	—	—
Sanfoin.....	—	—	—	—	—	—
Soybean 5.....	—	—	—	—	1:2560	—
Soybean 68.....	—	—	1:80	—	1:20	1:2560
Soybean 504.....	—	—	—	—	1:40	1:2560
Wistaria 421.....	—	—	—	—	—	—

by centrifugal force. The resulting serum was preserved with 0.2 to 0.3 per cent of tricresol. At this point the serum was stored in the ice box until needed.

The results obtained by the application of the two tests mentioned, shown in tables 2 and 3, compare favorably. It will be noted, however, that in certain cases where cultures representing different cross-inoculation groups are

compared, the cross-agglutination test was negative, whereas a  $\pm$ , +, ++, or even a +++ was obtained in the complement-fixation test. Cowpea 600 gave ++ with the antiserum of clover 5, and lupines 3 and 16 each gave a +++ complement-fixation, with soybean culture 504, but showed negative agglutination with the same antisera. In most other cases, however, the data in one table check with those in the other. Bean culture 17, for example, and bean culture 80, both of the same cross-inoculation group, show good comparative results. The former culture gave good agglutination with a 1:20 dilution of the antiserum of the latter. The corresponding complement-fixation test showed a ++ result. It may be noted that a few extra cultures were added to the list of antigens for agglutination. These tests are of significance in work of this nature, since they aid in the separating of nodule-bacteria of low nitrogen-fixing ability in a given cross-inoculation group from the more effective nitrogen-fixers of the same group.

#### PROTEIN KINSHIP OF CROTALARIA SPECIES

Baldwin, Fred, and Hastings (1) have shown a fair correlation between protein-kinship of legumes and cross-inoculation groups. Since the *Crotalaria* species investigated in this work are of special interest and seem to interchange nodule bacteria among themselves and with the cowpea, their protein relationship is of interest. Seeds from only 15 of the 22 species investigated were available, however, so this survey could not include the entire list. Alsike clover and the field pea, each belonging to separate cross-inoculation groups and different from the cowpea group, were tested by way of comparison with *Crotalaria* species.

In the carrying out of these tests, healthy rabbits were immunized to saline extracts of the ground seeds of alsike clover (*Trifolium hybridum*), cowpea (*Vigna sinensis*), *Crotalaria mundyi*, and the field pea (*Pisum sativum*). The seeds were treated with 70 per cent alcohol for 5 minutes, washed, and ground in a sterile mortar. This macerated material was mixed with approximately 4 volumes of saline and allowed to stand over night in the refrigerator. The supernatant fluid was decanted into sterile test tubes and preserved with tricersol.

The rabbits were given four intraperitoneal injections of 3 cc. of the seed infusion at intervals of 5 days. Seven days after the last injection, they were bled and the antisera collected as described for the complement-fixation tests. The antigens (seed infusions) for carrying out the precipitin tests were filtered and diluted to a point where very little turbidity remained.

The precipitin tests were carried out by the contact method in capillary tubes, the antisera being drawn into the tube, the constricted portion sealed, and the antigen slowly added to form a stratum on the serum. The tests were then incubated 30 to 40 minutes at 37°C. and read. A white ring formed at the point of junction of the two liquids, indicating a positive reaction.

The results of these precipitin tests are recorded in table 4. The density of the precipitate formed by the antiserum with its specific antigen in each case was taken as a standard and indicated by the "four plus" (++++) sign. The other results were given one, two, three, or four plus signs according to the amount of precipitate formed as compared to the standard. The negative sign was used to indicate no precipitate.

These data would indicate in general a close degree of protein kinship among the *Crotalaria* species tested and, in most cases, with the cowpea. In the case of *Crotalaria lanceolata*, *polysperma*, *spectabilis*, and *verrucosa*, the precipitate with the cowpea antiserum was slight or none. These tests were repeated with

TABLE 4  
Results of the precipitin test for antisera and 15 species of *Crotalaria*

ANTIGENS	ANTISERA			
	<i>C. mundyi</i>	Cowpea	Field pea	Alsike clover
<i>C. alata</i> .....	+++	++	—	+—
<i>C. anagyroides</i> .....	++++	++	—	—
<i>C. Axillaria</i> .....	+++	++	—	—
<i>C. goreensis</i> .....	++	+++	—	—
<i>C. incana</i> .....	++++	+++	—	—
<i>C. lanceolata</i> .....	++++	+—	—	—
<i>C. mundyi</i> .....	++++	+++	±	—
<i>C. mysorensis</i> .....	++	+++	—	—
<i>C. polysperma</i> .....	++++	±	—	—
<i>C. retusa</i> .....	+++	++	—	—
<i>C. spectabilis</i> .....	++++	—	—	—
<i>C. striata</i> .....	+++	+	—	—
<i>C. valentonii</i> .....	+++	+++	—	—
<i>C. vallicola</i> .....	++++	+++	—	—
<i>C. verrucosa</i> .....	++++	—	—	—
Alsike clover.....	+	±	—	++++
Cowpea.....	—	++++	—	—
Field pea.....	—	—	++++	—

the same result, and are taken to indicate that only slight protein relationship exists between these *Crotalaria* species and the cowpea. The reactions of field pea and alsike clover antisera with nonspecific antigens appear to signify that little or no kinship exists between these two plants and the *Crotalaria* tested.

#### HYPOTHESIS OF GROUP INOCULATION

The phenomenon of cross-inoculation or group inoculation and the mode of infection have been known (11) and generally accepted for a number of years. The underlying reason for nodule formation by a given culture of *Rhizobia* on

the roots of a given leguminous plant, or a group of such plants, and for failure of this same culture to accomplish this, in case of other closely related plants, is not understood.

Agricultural bacteriologists generally seem to consider entrance of *Rhizobia* into the root a true infection as in a case of a parasite in disease. However, in the light of present-day knowledge of plant physiology and plant "make-shifts" in nitrogen utilization, it seems entirely possible that the leguminous plant possesses an enzyme which enables it to use the particular form of organic nitrogen contained in the respective symbiont. The fact that plants have some power of selecting is borne out by the works of Perkins (10) and Voorhees (20) with varieties of the soybean.

The indications that poor inoculation is obtained in the presence of sufficient nitrate nitrogen (for plant development), and that fluctuation of the calcium nitrogen ratio (12) may influence nodule formation, cause the writer to believe that if conditions are such as to enable the plant to use more nitrogen than is available in the soil, the plant becomes infected and uses the bacterial nitrogen. In addition to the aforementioned, certain plants, such as rice, prefer ammonia to nitrate nitrogen (9). Certain other green plants, Venus' fly trap, (*Dionaea muscipula*), sundew (*Drosera intermedia*), and others, are able to entrap insects and utilize the nitrogen. These odd nitrogen "makeshifts" are recognized and accepted by physiologists, and it seems reasonable to assume that the leguminous plant and its respective bacterial symbionts present another striking example of the same thing.

If the hypothesis mentioned should be correct, a question as to whether the plant uses any cycle-stage of the bacteria themselves or their excretions arises. Beijerinck, as well as Bewley and Hutchinson (2) and Thornton (18), describes at least three stages in the life cycle of several species of *Rhizobium*. Prazmowski (11) and others describe a sheath which surrounds the bacterium soon after the infection takes place. Since the coccoid stage (2) becomes motile and swims about (in cultures at any rate) without a sheath for a time, it may be that these unprotected forms are used by the plant. Figure 2 of plate 2 shows the stages in the life cycle of one of the cowpea nodule bacteria. Since the literature gives full discussions of older theories attempting to explain group-inoculation it need not be reviewed here.

#### SUMMARY AND CONCLUSIONS

Certain strains of nodule bacteria belonging to the cowpea cross-inoculation group show greater power of nitrogen-fixation than other strains in the group.

The complement-fixation test and the agglutination reaction were compared as methods for separating or classifying strains of nodule bacteria. They appear to be about equal in reliability, but because of its simplicity the agglutination reaction is to be preferred.

A close protein kinship has been shown to exist among the 15 *Crotalaria* species tested. Most of the species also happen to be related to the cowpea plant. The indication is that none of them is closely related to alsike clover or to the field pea.

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## PLATE 1

## EFFECT OF INOCULATION WITH VARIOUS SOYBEAN AND COWPEA CULTURES ON THE GROWTH OF SOYBEAN AND COWPEA PLANTS

FIG. 1. Efficiency of soybean and cowpea nodule bacteria in symbiosis with the cowpea plant.

- Pot 1—uninoculated control
- 2—inoculated with soybean culture 6
- 3—inoculated with cowpea culture 11
- 4—inoculated with soybean culture 915
- 5—inoculated with soybean culture 1
- 6—inoculated with cowpea culture 10
- 7—inoculated with cowpea culture 2

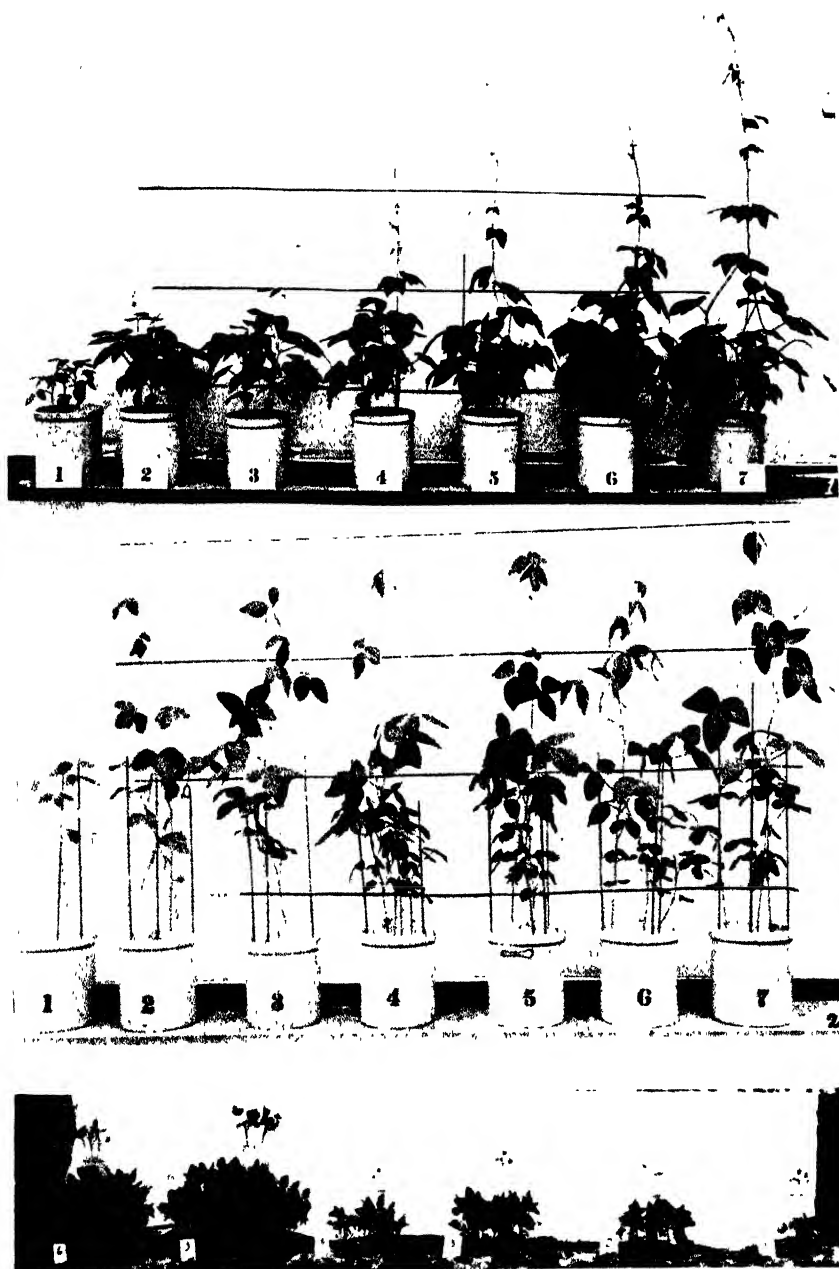
FIG. 2. Soybean plants showing variation in growth due to differences in nitrogen-fixing power of various cultures of soybean and cowpea nodule bacteria.

- Pot 1—uninoculated control
- 2—inoculated with cowpea culture 28
- 3—inoculated with soybean culture 6
- 4—inoculated with soybean culture 13
- 5—inoculated with soybean culture 1
- 6—inoculated with soybean culture 915
- 7—inoculated with cowpea culture 28

FIG. 3. Variation in growth due to the effect of different cultures on the cowpea plant.

The pot elevated behind the field test in each case shows the result of the greenhouse portion of the experiment. As is stated in the text, the high temperatures in the greenhouse retarded the development of the pot plants during the last 3 weeks of the test.

- Plat and Pot 1—uninoculated control
- 2—inoculated with soybean culture 5
- 3—inoculated with cowpea culture 9
- 4—inoculated with cowpea culture 31
- 5—inoculated with cowpea culture 60Q
- 6—inoculated with cowpea culture 23



## PLATE 2

FIG. 1. The well-inoculated roots of cowpea plants in small plat 5 shown in plate 1, fig 3.

FIG. 2. Morphological forms of cowpea nodule bacteria, culture 600 grown in asparagus-mannitol solution.

Photomicrograph 5—a dilute carbol fuchsin mount of a 48-hour culture—banded rods

6—nigrosin mounts of an 8-day culture—bacteroid stage

7—a nigrosin mount of a 17-day culture—coccoid stage

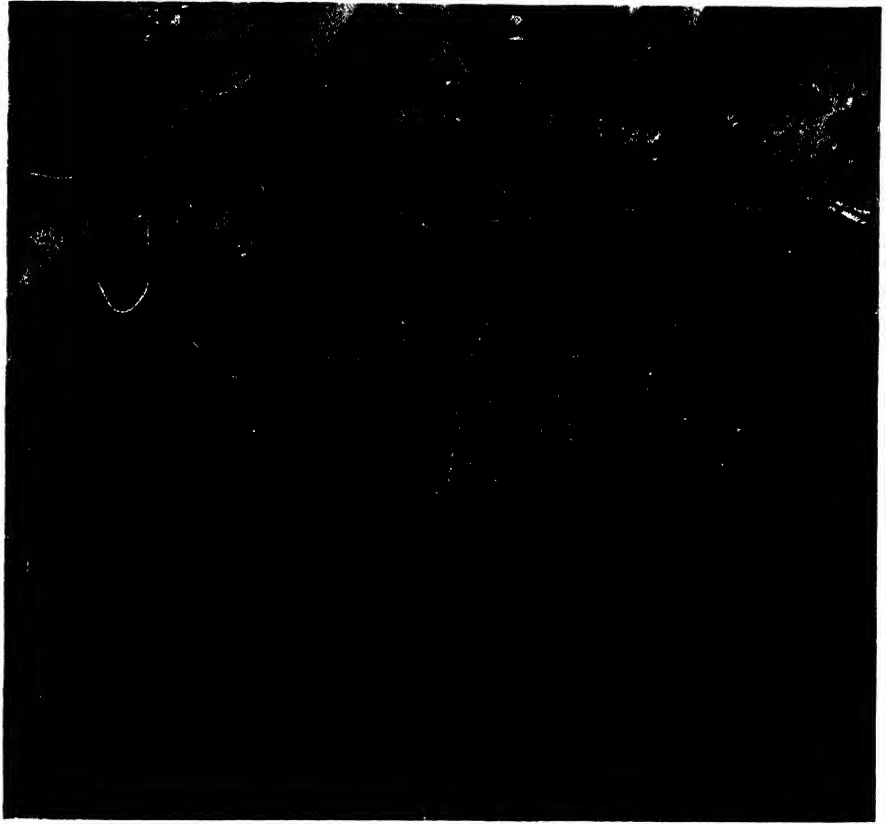
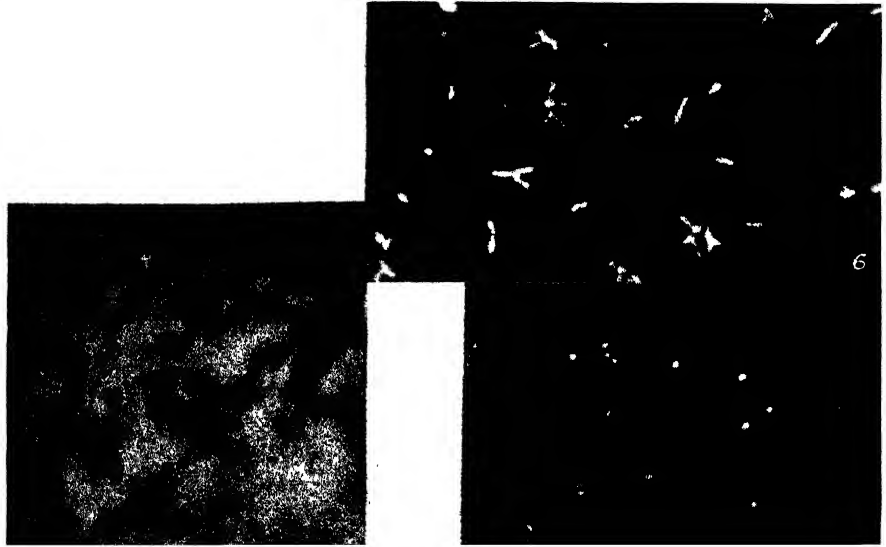


FIG. 1





# A MIGRATION METHOD FOR THE DETERMINATION OF REPLACEABLE BASES IN SOILS<sup>1</sup>

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In connection with studies of calcareous hardpans, it is often desirable to determine the ratio of the replaceable bases in the different horizons of the soil profiles under investigation. The common occurrence of calcium and magnesium carbonates in these soils makes it necessary to use some method not seriously affected by such salts but capable of strongly differentiating those soil conditions that are affecting crop production. In the course of such studies at this station, two commonly utilized methods and one new one have been investigated. The results obtained with the new method warrant calling especial attention to it. Indications are that this means of study may bring out new conceptions, especially with regard to the ratio of the replaceable bases to one another.

## REVIEW OF LITERATURE

Several methods for determining the replaceable bases in soils have been proposed. Kelley and Brown (5) and Chapman and Kelley (4) recommend the use of normal solutions of ammonium chloride or ammonium acetate, which are used to leach the soil successively. The use of such solutions requires the leaching of a small soil sample with relatively large amounts of solution, ratios of 1 part of soil to 40 parts of solution being quite general. In these methods the solubility of certain minerals is a disturbing factor for which corrections are difficult to make. The most common difficulty is the relatively great solubility of calcium and magnesium carbonates in solutions of ammonium salts. In certain cases, also, organic matter is dissolved, and the absorbing complex may be thereby altered in composition as the leaching proceeds.

Burgess and Breazeale (3) and Magistad and Burgess (10) have suggested methods for the determination of replaceable bases in calcareous soils. Burgess and Breazeale suggested the use of 0.1 *N* BaCl<sub>2</sub> solution for leaching the soil, and showed that there was negligible solubility of the alkaline earth carbonates in this solution. It seems possible, however, that the weaker solution would not react quite so strongly with the absorbing complexes, although Magistad and Burgess claim to have found that concentration made but little difference. Since we are dealing with a series of simultaneous equilibrium reactions, as is evident from the apparent need for successive leachings to complete them, it seems unusual if concentration plays no part. The effect of the concentration of one salt in solution upon the reaction of other substances in mixtures is usually important, and in replacement reactions in soils several salts are generally present at once. These authors suggested the use of alcoholic salt solutions for leaching. The dehydrating effect of alcohol on the colloidal complexes,

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which would undoubtedly alter their reactivity, makes such procedure appear too artificial to indicate what the soil behavior would normally be in an aqueous medium. Ultimate use of replacement data would normally be made in a soil-water system.

The use of electro dialysis (2) to determine the replaceable bases in soils would be extremely simple and satisfactory if there was a certainty that the character of the complex was not changed during the determination. The reaction changes so strongly that the pH value of the electro dialyzed soil often reaches a value of 2.5, and appreciable quantities of aluminum appear in solution. This aluminum may be derived from the breakdown of the colloidal complex itself, and the solubilities of many other components is undoubtedly increased. Such a drastic change in soil reaction must considerably upset the normal equilibrium under which the results of the determination are to be applied.

Two main difficulties in connection with present methods of determining replaceable bases must be overcome. One involves the elimination of the excessive solubility effects in leaching methods, and the second involves the elimination of artificial and drastic changes in the complex itself during the extraction.

In 1923 Kendall and Crittenden (6), and in 1924 Kendall and White (7) made separations of the isotopes of chlorine by means of an ionic migration method. The following year certain rare earths were separated by the same procedure by Kendall and Clarke (8). The method is essentially one in which the substances to be separated are dissolved and imbedded in a plug of agar in a cylindrical glass tube of suitable dimensions. Other electrolytes are mixed with agar and placed in contact with the agar plug containing the substances to be examined. It is necessary to have a faster moving ion in the agar ahead of the plug to be examined, and a slower moving one following, in order to preserve the sharp boundaries of the original material. One colored ion may be employed to indicate the course of the migration. Thus, if calcium and magnesium were in the original plug of agar, it would be necessary to use some cation with a higher mobility between them and the cathode toward which they will migrate. Following them should be a slower, colored cation. The concentrations best adapted for leading and following ions are suggested by the relation  $\frac{C}{T} = \frac{C'}{T'}$ , where  $C$  and  $C'$  = equivalent concentrations and  $T$  and  $T'$  = transference numbers of the cations.

An attempt has been made to use this method for the extraction of replaceable bases in soil. It was found that the ammonium ion is a relatively fast moving cation exceeding all of those in the soil except H ion. It can thus be used for replacement, and lends itself readily for use as the leading ion during migration. Cobalt ions, colored red, are slower moving than the soil bases and make excellent following ions.

The absolute ionic velocities of the bases we are interested in, at a temperature of 18°C., and at unit potential gradient, are as follows:

	cm. per second
Hydrogen.....	0.003242
Ammonium.....	0.000668
Potassium.....	0.000665
Calcium.....	0.000534
Magnesium.....	0.000471
Sodium.....	0.000456
Cobalt.....	0.000420*

\* Determined in 2 per cent agar at a potential gradient of about 2 volts/cm. Value only approximate.

## EXPERIMENTAL METHODS

### *Methods of replacement*

*Tenth-normal barium chloride method.* According to the directions of Burgess and Breazeale, 100 gm. of soil is placed in a glass percolating tube and

leached with 700 cc. or more of 0.1 *N* BaCl<sub>2</sub> solution until free from replaceable calcium. The percolate is then filtered, if necessary, and made up to a standard volume, from which aliquot samples are taken for analysis. The details of the special methods suggested to eliminate the excessive quantities of barium salts need not be mentioned. In this work, the standard methods suggested by the authors were employed.

*Normal ammonium acetate method.* Ten-gram samples of oven-dry soil are weighed into 400-cc. Erlenmeyer flasks, and 250 cc. of normal ammonium acetate solution is added to each. The flasks are kept at a temperature of 70°C. on top of a steam bath for about 15 hours and then their contents are poured on a filter and washed three times with 50-cc. portions of hot extracting solution, finally making the volume of leachate exactly 400 cc. Aliquots of this solution are then taken for analysis. It was found in a separate experiment that 400 cc. of a saturated solution of CaCO<sub>3</sub> in normal ammonium acetate solution contained 297.6 mgm. of Ca, which was used as a correction figure whenever the soil sample contained carbonates.

*Migration method.* Ten grams of oven-dry soil is weighed out, in a glass weighing scoop, and 10 cc. of hot normal ammonium acetate added slowly, the entire soil mass being carefully saturated. The mixture is allowed to stand about 1 hour. Meanwhile, a glass tube about 2.5 cm. in diameter and 45 cm. long is stoppered at one end, and melted 2 per cent agar in normal ammonium acetate is poured in. When solid, the column of agar is trimmed to a length of 25 cm. A wash bottle containing hot 2 per cent agar in normal ammonium acetate is used to wash the soil from the glass scoop into the cylinder. The length of the agar-soil plug should not be greater than 8 cm. When this is cooled, a hot 2 per cent agar solution, made up with 0.5 *N* cobalt acetate, is used to fill the cylinder. When cool, the end of the cobalt agar may be cut off to expose a clear bubble-free surface. The tube now consists of 25 cm. of ammonium acetate agar on one end, 8 cm. or less of soil-ammonium acetate agar in the center, and 12 cm. of cobalt acetate agar on the other end.

Two sets of two rubber stoppers on short pieces of glass tubing of about 1.5 cm. diameter are placed top to top with the tubes extending only to the ends of the stoppers. These are inserted into the ends of the cylinder containing the agar and should support the agar column firmly at each end. On each end of the cylinder a suction flask is now attached. In the flask, at the cobalt agar end, is placed a 0.5 *N* solution of cobalt acetate, into which a platinum electrode is inserted through the suction tip of the flask. Into the other flask is placed a normal solution of copper acetate and a copper electrode is inserted through the suction tip. The two electrodes are connected with a direct current generator, so that the copper electrode becomes the negative electrode. This insures the migration of the cations into the end of the tube containing ammonium acetate agar. The whole set-up may be supported by ring stands and clamps. Figure 1 illustrates the essential points in the experimental arrangement.



A current of about 110 volts and 0.5–0.6 amperes is passed through the tube until cobalt ions appear at the other end of the soil-agar plug. This requires from 5–6 hours. At the end of this time all faster moving free ions are out of the soil. The electrode flasks are disconnected, and the ammonium acetate agar plug containing the cations which have been replaced in the soil and have migrated out of it, is cut into suitable lengths, placed in a 100-cc. quartz evaporating dish, and after a few drops of sulfuric acid have been added is evaporated to dryness in an oven at 105°C. Ignition at red heat in a muffle furnace leaves a white ash, which is dissolved in hot water and a few drops of hydrochloric acid, and the volume made up to 200 cc. or other convenient quantity. Aliquots of this solution can be taken for analysis of calcium, magnesium, sodium, and potassium.

Purified agar should be employed in this procedure, but even the best quality material contains appreciable amounts of the bases also present in the soil. A blank determination of base content, using a column of normal ammonium acetate agar of the same length and from the same tube that will be used in the

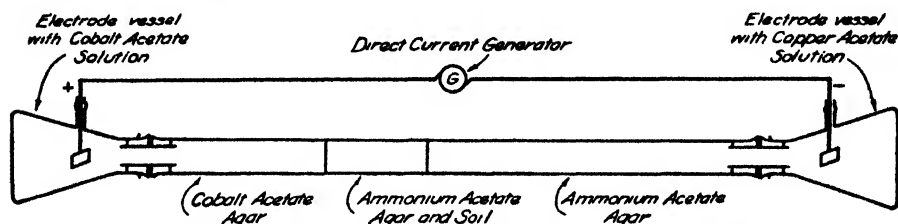


FIG. 1. EXPERIMENTAL ARRANGEMENT FOR EXTRACTING THE BASES FROM SOILS BY THE MIGRATION METHOD

soil studies, should be made. In the studies here reported it was found that the agar column contained 11.2 mgm. Ca, 6.12 mgm. Mg, 142 mgm. K, and 3.76 mgm. Na. These values are subtracted from the values obtained in the regular analysis after migration.

#### *Methods of analysis*

**Calcium.** The method outlined by Burgess and Breazeale was used to determine this element in extracts obtained by their method. In the other methods, official volumetric methods were used.

**Magnesium.** The method of Burgess and Breazeale was used for extracts made according to their method. In other studies, official gravimetric methods were employed.

**Potassium.** The sodium cobalti-nitrite method of Kramer (9) with certain modifications was used throughout these studies, titrations being made with 0.1 *N* potassium permanganate. The barium salts in the Burgess-Breazeale method were removed in accordance with directions given by them.

**Sodium.** The method of Barber and Kolthoff (1) utilizing the zinc uranyl acetate reagent was used, and the precipitate dried and weighed. A barium-

free solution was obtained in the same manner as used in the determination of potassium.

## SOILS

Ten soils were selected for the preliminary comparison of methods. Four contain carbonates and are strongly alkaline; three are slightly alkaline or

TABLE 1

*Characteristics of the soils used in this investigation*

LOCATION OF SOIL	TEXTURE	COLLOIDAL CON- TENT	pH VALUE	QUAN- TITY OF CARBO- NATES	OTHER CHARACTERISTICS
		<i>per cent</i>		<i>per cent</i>	
Prosser 1	Fine sandy loam	18	8.60	1.44	Arid—sage brush soil
Prosser 2	Fine sandy loam	19	8.57	0.75	Arid—sage brush soil
Prosser 3	Fine sandy loam	18	8.40	0.81	Arid—sage brush soil
Colton	Silt loam	31	7.25	....	Semi arid—prairie soil
LaCrosse	Silt loam	30	7.12	....	Semi arid—prairie sage
Washtucna	Fine sandy loam	20	7.65	....	Arid—sage brush soil
Woodland	Clay loam	32	4.70	....	Humid—residual forested
Albion	Silt loam	26	5.85	....	Semi arid—prairie soil
Walla Walla 1	Silt loam	22	6.10	....	Semi arid—prairie soil
Walla Walla 2	Hardpan	..	9.00	7.92	Sage—White alkali land

TABLE 2

*Milliequivalents of replaceable bases per 100 gm. of soil as determined by the method of Burgess and Breazeale using 0.1 N BaCl<sub>2</sub> solution*

SOIL	MILLIEQUIVALENTS PER 100 GM. OF SOIL				
	Calcium	Magne- sium	Potassium	Sodium	Sum
Prosser 1.....	11.78	2.06	0.26	1.63	15.73
Prosser 2.....	11.92	1.78	0.18	1.76	15.64
Prosser 3.....	11.66	1.68	0.19	2.08	15.61
Colton.....	18.00	5.63	0.61	0.34	24.58
LaCrosse.....	11.25	3.80	0.99	0.39	16.43
Washtucna.....	10.00	2.90	1.10	0.42	14.42
Woodland.....	10.50	4.75	0.58	0.22	16.05
Albion.....	14.55	3.00	0.38	0.61	18.54
Walla Walla 1.....	11.95	2.33	0.75	0.27	15.30
Walla Walla 2.....	7.62	3.27	1.85	2.98	15.72

faintly acid; and two are strongly acid in reaction. In texture they vary from fine sandy loam to clay loam. One sample consists of calcareous hardpan material. Table 1 gives some of the more important characteristics of the samples.

## EXPERIMENTAL RESULTS

*Tenth-normal barium chloride method*

The results obtained when 100 gm. of soil is leached with successive 50-cc. portions of 0.1 *N* BaCl<sub>2</sub> until a volume of about 700 cc. is obtained are given in table 2. From 65-78 per cent of the sum of the bases analyzed consists of calcium, and this element with magnesium makes up the greater part of the total. The amount of replaceable sodium exceeds the magnesium in only one case, and is usually considerably smaller. The quantities of exchangeable potassium are small, varying from about 1 to 11 per cent of the total replaceable bases analyzed. The method indicates that the alkaline earth bases are dominant in all soils except the hardpan material. The Woodland sample is a strongly leached residual soil derived from basaltic rocks, and it would appear that the method does not differentiate this strongly enough to show its marked

TABLE 3

*Milliequivalents of replaceable bases per 100 gm. of soil as determined by the method of Chapman and Kelley using normal ammonium acetate solution*

SOIL	MILLIEQUIVALENTS PER 100 GM. OF SOIL				
	Calcium	Magne- sium	Potassium	Sodium	Sum
Prosser 1.....	46.62	4.91	1.00	1.09	53.62
Prosser 2.....	35.12	5.55	1.27	1.10	43.04
Prosser 3.....	25.62	5.64	1.63	1.08	33.97
Colton.....	19.25	6.28	1.45	1.01	27.99
LaCrosse.....	12.50	4.27	1.81	0.88	19.46
Washtucna.....	12.75	4.01	2.57	0.93	20.25
Woodland.....	11.75	6.19	1.18	0.91	20.03
Albion.....	16.50	4.91	1.05	0.91	23.37
Walla Walla 1.....	15.75	5.17	1.09	0.76	21.07
Walla Walla 2.....	93.12	18.93	1.81	7.40	121.26

difference in character from the rest of the samples. The Colton sample is more severely leached than the LaCrosse sample and contains about the same amount of colloidal material; yet more alkaline earth bases are shown by this method in the Colton soil. The results do not quite provide a logical basis for the explanation of the behavior of these soils in the field, and this may be the result of a tendency to replace more completely the alkaline earth bases than the alkali bases. The work of Burgess and Breazeale shows that this 0.1 *N* BaCl<sub>2</sub> solution does not directly dissolve appreciable amounts of calcium, and yet proportionally large amounts of the totals replaced and analyzed are made up of calcium and magnesium.

*Normal ammonium acetate method*

The results obtained with the normal ammonium acetate method, when carried out in the manner already described, are shown in table 3. These data

show a much greater energy of replacement, as can be noted by observing the totals analyzed in this and the preceding table. But again the alkaline earth bases are shown to be dominant, and in some cases their importance is undoubtedly greatly exaggerated, on account of solubility effects which cannot be entirely corrected. Thus, from 58-86 per cent of the total bases found by analysis consist of calcium alone, and in many cases over 90 per cent of the total is alkaline earth bases. The part that sodium and potassium play in the field behavior of these soils is masked by the solubility of the calcium and magnesium, especially in the case of calcareous soils.

### *Migration method*

Table 4 contains the data obtained with the migration method in which the replaceable bases are extracted after a single treatment with normal ammonium

TABLE 4

*Milliequivalents of replaceable bases per 100 gm. of soil as determined by the migration method, using normal ammonium acetate as replacing solution*

SOIL	MILLIEQUIVALENTS PER 100 GM. OF SOIL				
	Calcium	Magne- sium	Potassium	Sodium	Sum
Prosser 1.....	13 44	4 82	1.70	5.79	25.75
Prosser 2.....	12 64	4 65	1.01	4 58	22 88
Prosser 3.....	11 44	3 66	0 81	4 49	20 40
Colton.....	12.94	8 95	1 36	2 46	25 71
LaCrosse.....	12 44	9 05	1 54	2 81	25.84
Washtucna.....	13 69	9 68	1 80	2.28	27.45
Woodland.....	7 44	3.65	1 35	3.16	15.60
Albion.....	12 77	8 65	1 78	3 14	26 34
Walla Walla 1.....	8 69	4 49	1 36	1.77	16.31
Walla Walla 2.....	13 44	7 77	2.49	10.88	34.58

acetate solution. There is a general agreement between the results of the Burgess and Breazeale method and this one, with respect to calcium, although sometimes larger and sometimes smaller amounts are obtained with the migration method. In all except one case, more magnesium is obtained by the migration method than by the 0.1 N BaCl<sub>2</sub> method. Generally the magnesium obtained by the normal ammonium acetate method and by the migration method are comparable, sometimes one and sometimes the other being greater. The most striking difference shown by the migration method is the relatively strong replacement of potassium and sodium, especially the latter. In every soil the replaceable sodium obtained through migration procedure exceeds that obtained by the leaching methods. This makes the results of this method especially valuable in the study of alkali soils and hardpan formation, because it shows the influence of the alkali bases and offers a suggestion for the field behavior of these soils, which is not brought out by other methods. Why the

leaching methods fail to extract as much sodium as the migration method can only be suggested, but some preferential solution process may inhibit the sodium release and stimulate the calcium and magnesium release. The migration method apparently indicates more clearly the true ratio of the various replaceable bases, for it is not involved by abnormal solubilities as a result of leaching and yet it is capable of obtaining from the soil complexes at least the same amounts of both calcium and magnesium as can be obtained by the other two methods. Moreover, it has been found that the pH values of the soils after the migration procedure are about the same as those obtained on the original samples.

### *Ratios of the replaceable bases*

A clearer picture of the ratios of the replaceable bases to one another can be obtained by a study of the material presented in table 5. The ratio of alkali

TABLE 5

*A comparison of the replaceable base ratios in the soils treated by the different methods indicated*

SOIL	Ca + Mg Na + K RATIOS			Ca Mg RATIOS		
	0.1 N BaCl <sub>2</sub>	N NH <sub>4</sub> Ac	Migration	0.1 N BaCl <sub>2</sub>	N NH <sub>4</sub> Ac	Migration
Prosser 1.....	7.32	24.65	2.43	5.71	9.49	2.78
Prosser 2.....	7.06	17.16	3.09	6.69	6.32	2.71
Prosser 3.....	5.87	11.53	2.84	6.94	4.54	3.12
Colton.....	24.87	10.37	5.73	3.19	3.06	1.44
LaCrosse.....	10.90	6.23	4.94	2.96	3.11	1.37
Washtucna.....	8.48	4.78	5.72	3.44	3.17	1.41
Woodland.....	19.16	8.58	2.45	2.21	1.89	2.03
Albion.....	17.92	10.92	4.35	4.60	3.36	1.47
Walla Walla 1.....	14.00	10.38	4.21	5.12	3.04	1.93
Walla Walla 2.....	2.25	12.16	1.58	2.34	4.92	1.72

to alkaline earth bases in exchangeable form as determined by the different methods varies between wide limits. The average ratio, for instance, for all 10 soils when the bases were exchanged through the medium of 0.1 N BaCl<sub>2</sub> solution is 11.78; for the bases obtained by leaching with normal ammonium acetate solution, 11.67; and for the migration method, 3.73. This shows clearly the tendency for the migration method to extract a larger proportion of the alkali bases than is possible by other methods. At the same time the migration method extracted amounts of alkaline earth bases comparable to those obtained by the other methods. The fact that sodium is extracted so readily by the migration method and does not appear in the extracts in the leaching methods, is significant and seems to indicate a weakness in these methods, in so far as alkali soil studies or hardpan studies are concerned or any investigation of soils where the ratio of sodium and calcium is considered significant. For such studies the migration method is peculiarly suited.

The migration method also favors the extraction of magnesium, as is shown by the calcium-magnesium ratios in table 5. In all but one soil, more magnesium in proportion to calcium was obtained through the use of the migration method than by the other methods. From the physiological standpoint this should be significant, and if any relationships between replaceable bases and plant growth are to be established it would appear that the migration method would give a truer picture of the nutrient ratios than certain other methods. It seems strange that with a single treatment with normal ammonium acetate solution, followed by migration, larger amounts of both sodium and magnesium are usually obtained than can be liberated through leaching processes with the same solvent. It is possible, however, that the presence, in many cases, of excessive amounts of calcium which is dissolved directly from the soil forces back the reactions by which the magnesium and sodium are coming into solution. This would tend toward abnormally high calcium yields and a repression in magnesium and sodium yields, not because the latter elements are not in replaceable form, but because they are unable to go into solution in the presence of so much calcium. If the indications given by the use of the migration method are further substantiated, it would seem that the earlier pictures of the relationship between replaceable bases in the soil have not been entirely true, especially in calcareous soils.

#### *Other possible applications of the migration method*

In this study only the cations that migrate out of the soil have been investigated, but, by a suitable change in experimental procedure, it would be equally possible to study the anions. A slow moving, colored anion and a reversal of poles would enable the investigator to obtain data equally well for anion relationships. The relationship between soil bases and those absorbed by and present in the cells of plants could also be investigated by this method, by embedding ground plant tissue in agar and subjecting it to the usual migration procedure. Further studies along these lines are in progress.

#### SUMMARY

Three methods for the extraction of replaceable bases from soils have been compared on a group of 10 soils, 4 of which contain carbonates. The methods were the 0.1 *N* BaCl<sub>2</sub> leaching method of Burgess and Breazeale, the normal ammonium acetate leaching method of Chapman and Kelley, and a newly devised migration method for extraction after treatment of the soil with hot normal ammonium acetate solution.

The results of the comparative studies show a preferential extraction of alkaline earth bases over alkali bases in the case of the leaching methods, if the results obtained by migration procedure are considered as a basis for judgment. Since the migration method does not appreciably change the pH value of the soil during extraction, or bring about excessive solubilities through leaching, it seems to do away with the worst features of other methods, including electrodialysis.

The results of the migration method show that the present ideas, based upon leaching methods and others, regarding the ratios of replaceable bases in soils must be revised. There is generally more sodium and magnesium in replaceable form in the soils than has formerly been obtained through the use of the usual methods of extraction.

The significance of these newer relationships between exchangeable cation may more clearly show the reasons for the field behavior of soils and plants, especially in the drier climates, where carbonates often occur in the soils.

Use of the migration method may probably be made in connection with anion studies in soils and in the extraction of mineral constituents from plant tissues.

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# ELECTRODIALYSIS AS A MEANS OF STUDYING THE NATURE OF SOIL PHOSPHATES<sup>1</sup>

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Recently, electrodialysis has come into use as a means of studying the solubility and availability of the nutrient elements of soils. Varying opinions are held as to its value for the study of the nature of soil phosphates, and it was the purpose of this investigation to obtain further information along this line.

For a review of the literature, the reader is referred to the recent articles of Harper (3), McGeorge (5), and Scarseth (7).

The variables, mechanism, and interpretation of electrodialysis are best understood by a consideration of Hyman's (4) mathematical expression for the rate of desalting of the center compartment of a three compartment electrodialysis cell. This expression shows that time, potential gradient, and nature and concentration of substance to be electrodialyzed are important factors that must be given due consideration in the interpretation of results. In his treatment of the subject he assumed that the following individual phenomena affect the desalting: dialysis, electrolysis, electro-osmotic flow.

The expression given for the rate of desalting by electrodialysis is:

$$-\frac{dC}{dt} = \frac{q}{V} \times \frac{E}{F} \times \alpha(u + v) C \quad (A)$$

where:

$C$  = concentration of electrolyte within the membrane, expressed in millimoles.

$t$  = time.

$q$  = area of electrode.

$V$  = volume of suspension in center compartment.

$E = \frac{e}{l}$ ;  $e$  = potential on electrodes,  $l$  = distance between electrodes.

$F$  = 96,500 coulombs.

$\alpha$  = degree of dissociation.

$u + v$  = Hittorf's transference numbers.

<sup>1</sup> Contribution from the department of soils, University of Wisconsin, Madison, Wisconsin. Part I of a thesis submitted to the faculty of the University of Wisconsin in partial fulfillment of the requirements for the degree of doctor of philosophy. Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

<sup>2</sup> The writer wishes to express his appreciation for the helpful suggestions and criticisms tendered by Prof. E. Truog.



## EXPERIMENTAL

*Method of electrodialysis*

*Apparatus.* The apparatus used was a cell of the type devised by Mattson (6). The dimensions of the graphite anode and of the copper gauze cathode were  $5\frac{1}{4}$  by  $3\frac{3}{4}$  inches. Both the anode and cathode membranes consisted of parchment paper. No attempt was made to eliminate the effect of the electric charge of the membrane.

*Procedure.* During the course of this investigation, two types of materials were electrodialyzed; namely, phosphate compounds and soils. In electrodialyzing soils, 100 gm. of 60-mesh soil and 100 cc. of water were stirred together to form a suspension, which was then immediately transferred to the center compartment of the cell. The two outside compartments were each filled with 225 cc. of distilled water, and the electrodes connected to a source of direct current giving 75 volts. During the electrodialysis the voltage was regulated so that it was constantly 75 volts, but the amperage was allowed to drop as the resistance of the cell increased. At approximately 1, 3, 6, 12, 24, 36, 48, and 60 hours, samples were taken by draining the liquid out of the two outside compartments. The phosphate content was determined in the anode liquid by taking an aliquot, adding 2 cc. of 10 per cent magnesium nitrate solution, evaporating to dryness, igniting, dissolving the residue, and determining the phosphate colorimetrically, using Truog and Meyer's (9) modification of Denigés' method. It was necessary to ignite because the graphite anode slowly decomposed, forming a colloidal solution of carbon. The cathode liquid was titrated with 0.1 *N* HCl in order to determine the total bases removed.

The electrodialysis of phosphate compounds was carried out by mixing a weighed sample with 100 cc. of water and then transferring the suspension to the center compartment of the cell. From this point, the procedure was the same as in the case of soils.

*Electrodialysis of readily soluble phosphate compounds*

*Electrodialysis of tribasic calcium phosphates.* In view of the fact that calcium is usually the dominant active base present in most soils, it was assumed that the calcium phosphates would be the most important of the readily soluble phosphate compounds. Therefore, two of the more insoluble calcium phosphates; namely, tricalcium and rock phosphates, were electrodialyzed in order to have some basis of comparison. The results of this test are shown in figure 1.

These curves show that the rate at which the phosphate was removed was dependent to some extent upon the original concentration of the material present in the center compartment. The last traces of the phosphate were removed at a much slower rate.

*Electrodialysis of soils to which phosphates had been applied.* Attention was next turned to determining the manner in which phosphate is removed from soils to which calcium phosphates had been added. For the purposes of this

study a set of soils from the University of Illinois experimental field at Aledo<sup>8</sup> were used. A summary of the results obtained on the electro dialysis of these soils is given in table 1. Electro dialysis reveals surprising differences in the effect of different treatments. In the cases of the superphosphate and rock phosphate plats a large percentage of the phosphate had been converted into a form which was not attacked by electro dialysis. However, in the case of the lime-rock phosphate plat, none of the applied phosphate had been converted into a form from which electro dialysis would not readily remove the phosphate.

The rate curves for the electro dialysis of these soils (fig. 2) show that a large portion of the phosphate is removed at a constant rate, as is indicated by the

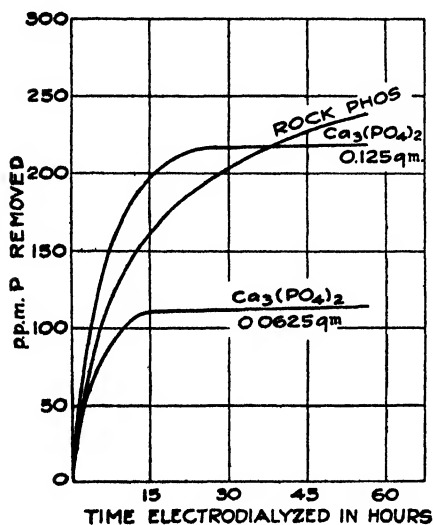


FIG. 1. RATES OF REMOVAL BY ELECTRODIALYSIS OF PHOSPHORUS FROM TRIBASIC CALCIUM PHOSPHATES AND ROCK PHOSPHATE

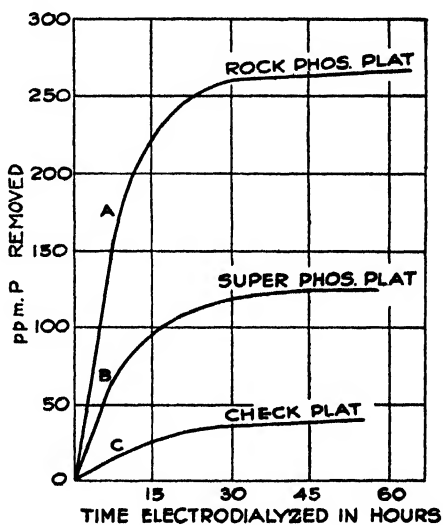


FIG. 2. RATES OF REMOVAL BY ELECTRODIALYSIS OF PHOSPHORUS FROM SOIL UNTREATED AND TREATED WITH CALCIUM PHOSPHATES

straight lines at the beginnings of the curves, i.e., OA, OB, OC. If these straight lines may be interpreted to mean that the same compound, for the most part, is being acted upon during the intervals of time which these straight lines represent, it becomes possible to calculate a specific electro dialysis constant. Assuming that the only variable is the original concentration of the compound, equation (A) may be written as follows:

$$-\frac{dC}{dt} = KC \quad (B)$$

OR

$$\text{slope} = KC \quad (C)$$

<sup>8</sup> The writer is indebted to the University of Illinois for these samples, which were taken in the the fall of 1928 and kindly furnished by Mr. A. U. Thor.

Using the value of  $C$  represented by the points A, B, and C, the values of  $K$  were calculated. The results of these calculations are given in table 2. It may be deduced from these calculations that about 50 per cent of the phosphate applied as fertilizer to these soils is present in them in the same or a similar

TABLE 1

*Removal of phosphorus during 50 hours of electro dialysis from soils of variously treated plats of Aledo experimental field, University of Illinois*

TREATMENT	PHOSPHORUS APPLIED PER ACRE (APPROXI- MATE)	PHOSPHORUS PER ACRE REMOVED BY ELECTRODIALYSIS		APPLIED PHOSPHORUS NOT RECOVERED BY ELECTRO- DIALYSIS
		Total	Total less check	
	pounds	pounds	pounds	per cent
Check.....	0	77	....	..
Superphosphate 4,066 pounds.....	293	250	173	59
Rock phosphate 8,334 pounds.....	1,042	526	449	43
Rock phosphate 8,334 pounds, lime 6 tons.....	1,042	1,202	1,125	..

TABLE 2

*The specific rate of electro dialysis,  $K$ , for soils from the variously treated plats of Aledo experimental field, University of Illinois*

TREATMENT	$c$	$-\frac{dC}{dt}$ SLOPE	$K$
Check.....	25	2.05	0.082
Superphosphate.....	70	8.24	0.118
Rock phosphate.....	170	20.0	0.118
Rock phosphate and lime.....	425	50.0	0.118

form, and that none of the phosphate in the check plat is in as soluble a form as this.

#### *Electrodialysis of difficultly soluble phosphate compounds*

*Electrodialysis of iron and aluminum phosphates.* What is the effect of electro dialysis upon difficultly soluble phosphate compounds? For the purposes of this study, samples<sup>4</sup> of ferric phosphate, aluminum phosphate, and duftenite [ $\text{Fe}_2(\text{OH})_2\text{PO}_4$ ] each containing 0.025 gm. of phosphorus were electro dialyzed. The results are shown graphically in figure 3. It may be seen from these curves that the compounds contained slight amounts of easily soluble phosphate as impurities as indicated by the curved part at the beginning. After these impurities were removed, the rate curves became straight lines except in the case of the ferric phosphate, which had a break in the straight line. In view of the fact that the original amount of phosphorus was the same in all cases, the

<sup>4</sup> The ferric and aluminum phosphates used were prepared in this laboratory by Mr. L. M. Weyker. They were considerably purer than ordinary commercial products obtainable.

slopes of the straight lines bear a simple relation to the rate of solution or hydrolysis of these compounds. It was noted that when the iron phosphates were being electrodialyzed, no iron was detected in the cathode compartment.

*Electrodialysis of soils containing only difficultly soluble phosphates.* For the purposes of this study two stock soils from the Experiment Station of the Association of Hawaiian Pineapple Canners were used. These soils are distinctly lateritic and contain a normal amount of total phosphorus which is practically all in difficultly soluble form. The results of the electrodialysis of these soils are shown in figure 4. The only generalization that can be drawn from these curves is that in the electrodialysis of soils of this type under these conditions, the rate of removal of phosphate from the iron and aluminum phosphates is

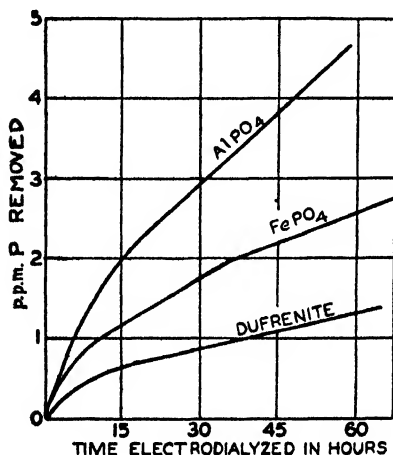


FIG. 3. RATES OF REMOVAL BY ELECTRODIALYSIS OF PHOSPHORUS FROM DIFFICULTLY SOLUBLE PHOSPHATE COMPOUNDS

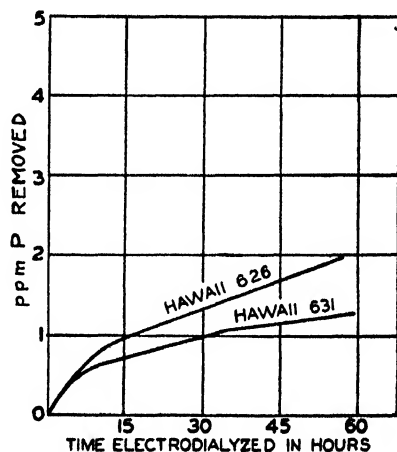


FIG. 4. RATES OF REMOVAL BY ELECTRODIALYSIS OF PHOSPHORUS FROM SOILS CONTAINING DIFFICULTLY SOLUBLE PHOSPHATE COMPOUNDS

extremely slow. It may be noted that in the electrodialysis of soils containing only difficultly soluble phosphates, the curves obtained are similar to those obtained with iron phosphates.

*The total quantity of phosphate removed from soils by electrodialysis*

A comparison of the total quantity of phosphate removed by electrodialysis and by three successive extractions, using a modification<sup>5</sup> of Truog's (8) method of determining readily soluble phosphorus is given in table 3. These data reveal a fairly good agreement between the amounts of phosphorus removed by electrodialysis and by three extractions with 0.002 *N* H<sub>2</sub>SO<sub>4</sub>. However, a

<sup>5</sup> The modification being that the ratio of soil to extracting solution was changed from 1 to 200 to 1 to 400.

critical examination shows that the soils may be divided into the following three classes:

Soils from which more phosphate is removed by acid extraction than by electro dialysis.

Soils giving a good agreement between the two methods.

Soils from which more phosphate is removed by electro dialysis than by acid extraction.

*Soils from which more phosphate is removed by acid extraction than by electro dialysis.* The lateritic soils of Hawaii, as far as studied, fall in the first class and are peculiar in that acid extraction removed more phosphorus than did

TABLE 3

*A comparison of the amounts of phosphorus removed from soils by electro dialysis for 60 hours, and by extractions with 0.002 N H<sub>2</sub>SO<sub>4</sub> of pH 3*

DESCRIPTION OR SOURCE OF SOIL	pH OF SOIL	P.P.M. OF PHOSPHORUS		
		Removed by electro-dialysis for 60 hours	Removed by 5 extractions with 0.002 N H <sub>2</sub> SO <sub>4</sub>	Amount by electro dialysis less amounts by extraction
Hawaiian soil 626.....	4.6	1.97	9.0*	-7.03
Hawaiian soil 631.....	4.4	1.28	9.0*	-7.72
Hawaiian soil 4391.....	6.3	50.0	376.0	-228.0
(Wisconsin) Superior clay.....	5.2	42.0	59.0	-17.0
(Wisconsin) Miami silt loam.....	5.8	9.2	28.0	-19.0
(Wisconsin) Miami silt loam..	6.7	96.0	112.0	-16.0
Aledo Field (Illinois), check platr..	5.0	39.0	41.0	-2.0
Aledo Field (Illinois), superphosphate plat..	5.0	125.0	112.0	+13.0
Aledo Field (Illinois), rock phosphate plat..	5.5	263.0	259.0	+4.0
Aledo Field (Illinois) lime and rock phosphate plat.....	6.0	601.0	599.0	+2.0
Montana soil.....	8.1	169.0	179.0	-10.0
Wyoming soil.....	7.5	199.0	114.0	+85.0
Idaho 10622.....	7.6	476.0	414.0†	+62.0
Idaho 10626.....	8.3	575.0	504.0†	+71.0

\* One extraction.

† Five extractions.

electrodialysis. A few of the middle western soils showed the same type of variation to a limited extent. The results obtained thus far lead one to believe that electro dialysis attacks calcium phosphates readily and iron and aluminum phosphates only sparingly. Consequently, it was assumed that acid extraction attacked difficultly soluble phosphates more readily than did electro dialysis.

In order to obtain a definite idea of the effect of 0.002 N H<sub>2</sub>SO<sub>4</sub> at pH 3 on iron and aluminum phosphates, 0.5-gm. samples were treated in exactly the same manner as 0.5-gm. soil samples were treated, the extraction method according to Truog (8) being used. The results of five successive extractions are shown in table 4. These results show that the ferric phosphate, dufrenite,

and soil 4391 were affected in somewhat the same manner with respect to removal of phosphate by the acid extractions, whereas the aluminum phosphate seemed to be affected in a somewhat different manner. These results have led to the belief that at pH 3, aluminum phosphate goes into solution, forming two soluble compounds; namely, aluminum sulfate and phosphoric acid, whereas the ferric phosphate forms one soluble and one less soluble compound; namely, phosphoric acid and a basic iron phosphate.

TABLE 4

*A comparison of the amounts of phosphorus dissolved in successive extractions with 0.002 N  $H_2SO_4$  of pH 3 from 0.5-gm. samples of difficultly soluble phosphate compounds and one soil*

MATERIAL	P.P.M. PHOSPHORUS REMOVED BY EXTRACTION WITH 0.002 N $H_2SO_4$				
	In first extraction	In second extraction	In third extraction	In fourth extraction	In fifth extraction
$AlPO_4$ .....	700	820	600	670	540
$FePO_4$ .....	260	160	92	80	68
Dufrenite.....	200	98	80	60	56
Hawaiian Soil 4391.....	180	120	76	68	54

TABLE 5

*The amounts of phosphorus removed in successive extractions with 0.002 N  $H_2SO_4$  of pH 3 from goethite treated with varying quantities of a soluble phosphate ( $KH_2PO_4$ )*

P.P.M. PHOSPHORUS ADDED TO 0.5 GM. SAMPLES OF GOETHITE	P.P.M. OF PHOSPHORUS REMOVED IN SUCCESSIVE EXTRACTIONS WITH 0.002 N $H_2SO_4$				PER CENT NOT REMOVED
	In first extraction*	In second extraction	In third extraction	In fourth extraction	
200	30	18	14	14	62
500	36	29	25	21	78
1,000	61	44	34	33	83
2,000	420	124	100	56	65
3,000	860	176	124	70	59
4,000	1,520	204	132	70	52
5,000	1,840	256	160	80	53

\* The first extraction contained some soluble phosphate left by the treatment.

If it is possible to obtain, by successive extractions, ferric phosphates which have decreasing solubilities, it should be possible to do the reverse, and build up phosphate compounds having increasing solubilities. Consequently, 0.5-gm. samples of Goethite ( $Fe_2O_3 \cdot H_2O$ ) were treated with increasing amounts of monobasic potassium phosphate ( $KH_2PO_4$ ) and 50 cc. of water, and allowed to go to dryness on the steam plate. These samples were then extracted with 0.002 N  $H_2SO_4$ . The results are given in table 5. In considering these data, the results obtained with the first extraction should be eliminated because some

of the phosphate removed was not derived from iron phosphates formed but from  $\text{KH}_2\text{PO}_4$  that had not reacted. The remaining results clearly show that a series of compounds with increasing solubilities had been formed.

In order to obtain further evidence of the nature of the phosphate present in soil 4391, a comparison of the rate of electro dialysis of this soil and of iron and aluminum phosphates was made. This is shown in figure 5. The curves for the iron and aluminum phosphates in figure 5 are based upon the data given in figure 3, and were calculated in the following manner: Since the curves given in figure 3 were obtained by the electro dialysis of samples containing 0.025 gm. of phosphorus whereas the sample of soil 4391 which was electro dialyzed contained 1.35 gm. of phosphorus, by simple proportion the slopes of the lines

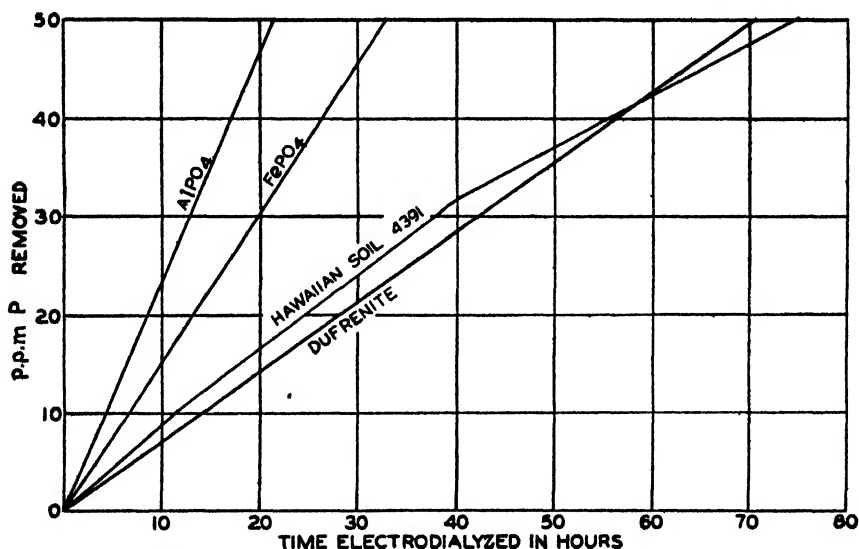


FIG. 5. A COMPARISON OF THE RATES OF REMOVAL OF PHOSPHORUS BY ELECTRODIALYSIS FROM HAWAIIAN SOIL 4391 AND FROM IRON AND ALUMINUM PHOSPHATES, ALL SAMPLES CONTAINING EQUAL QUANTITIES OF PHOSPHORUS

for the electro dialysis of samples of iron and aluminum phosphates, each containing 1.35 gm. of phosphorus, were calculated and expressed graphically as shown. These curves show that there is a marked similarity between the electro dialysis curves for soil 4391 and dufrenite.

In view of the fact that soil 4391 and dufrenite show a marked similarity in their behavior when they are either electro dialyzed or extracted with acid, it is believed that the main body of the phosphate present in this soil is in the form of dufrenite or in a form similar thereto. Also, it may be concluded that when more phosphate is removed from a soil by acid extraction than by electro dialysis for 60 hours under the conditions described, most of the phosphate present in that soil is in the form of iron phosphates.

*Soils giving a good agreement between the amounts of phosphate removed by*

*electrodialysis and acid extraction.* In the second class fall those soils from the humid temperate regions which contain a fair quantity of readily soluble phosphate. Data previously presented show that electrodialysis will remove this form of phosphate quite readily from soils. Consequently, when this type of soil is being dealt with, both methods appear to be equally satisfactory.

*Soils from which more phosphate is removed by electrodialysis than by acid extraction.* In the third class fall the calcareous or alkaline soils derived from the arid regions of the western part of the United States. On first consideration it might be thought that the reason for the difference found here might be

TABLE 6

*The amounts of phosphorus extracted by 0.002 N H<sub>2</sub>SO<sub>4</sub> of pH 3 from soils after being electrodialyzed for 60 hours, compared with the amounts extracted from the original soils in the third of successive extractions*

DESCRIPTION OR SOURCE OF SOIL	P.P.M. PHOSPHORUS EXTRACTED BY 0.002 N H <sub>2</sub> SO <sub>4</sub>	
	In first extraction of electrodialyzed soil	In third successive extraction of original soil
Hawaiian soil 626.....	8.0	.. *
Hawaiian soil 631.....	9 0	...*
Hawaiian soil 4391 .....	170.0	76 0
Superior clay—Wisconsin .....	21.0	11 0
Miami silt loam—Wisconsin.....	13 0	5 6
Miami silt loam—Wisconsin.....	18 0	12 0
Aledo Field, Ill.—Check plat.....	17.6	9 6
Aledo Field, Ill.—Superphosphate plat.....	18 0	16.0
Aledo Field, Ill.—Rock phosphate plat.....	24 0	32 8
Aledo Field, Ill.—Lime and rock phosphate plat.....	30 0	52 6
Montana soil.....	46 0	27 0
Wyoming soil .....	17 6	22.0
Idaho soil 10622.....	24 0	13 0†
Idaho soil 10626.....	52 0	34 0†

\* Not determined.

† Fifth extraction.

due to the calcium carbonate neutralizing the acid which was used for extraction. However, a study of the pH of the various acid extracts showed that in the case of the soils studied, only the pH of the first extract was affected. Because three to five acid extractions were made on these soils, it is believed that the neutralization of the acid by the calcium carbonate is not a plausible explanation of the differences obtained. Scarseth (7) also found that when calcareous soils are electrodialyzed, large amounts of phosphorus are removed.

The phosphorus-containing organic compounds of soils have not been thoroughly studied. It is here suggested that under alkaline soil conditions there may be organic compounds present in soils which yield phosphorus by electrodialysis but not by acid extraction.



*The nature of the difficultly soluble phosphates present in soils*

*Phosphates remaining in soils electrolyzed for 60 hours or extracted with acid.* The soils which had been electrolyzed for 60 hours to remove the readily soluble phosphate were dried at 40°C., pulverized to pass a 60-mesh sieve, and the solubility of the phosphate present was determined by extraction with 0.002 N H<sub>2</sub>SO<sub>4</sub>. The results of these determinations are given in table 6. The solubilities of the phosphate in practically all of the soils studied are of the same order. This would tend to indicate that the difficultly soluble phosphates in

TABLE 7

*Amounts of phosphorus recovered by extraction of soils and materials with 0.002 N H<sub>2</sub>SO<sub>4</sub> of pH 3 after these materials had been treated with 500 p.p.m. of phosphorus in form of KH<sub>2</sub>PO<sub>4</sub>*

SOIL OR MATERIAL TO WHICH 500 P.P.M. PHOSPHORUS WAS ADDED	P P M PHOSPHORUS EXTRACTED BY 0.002 N H <sub>2</sub> SO <sub>4</sub>				PER CENT APPLIED PHOSPHORUS NOT RECOVERED IN FOUR EXTRACTATIONS
	In first extraction	In second extraction	In third extraction	In fourth extraction	
Superior clay—after electrolysis.....	260	48	38	22	26.4
Miami silt loam—after electrolysis.....	300	50	44	22	16.8
Miami silt loam—after electrolysis.....	200	58	28	18	39.6
Montana soil—after electrolysis.....	330	60	33	18	11.8
Aledo Field, Ill.—after electrolysis.....	290	68	47	22	14.6
Idaho 10622—after electrolysis.....	320	62	45	22	10.2
Hawaiian soil 631.....	52	24	18	17	77.8
Hawaiian soil 626.....	30	17	13	13	84.4
Hawaiian soil 555.....	56	35	24	18	73.4
Hawaiian soil 1591.....	112	54	41	30	52.6
Hawaiian soil 1594.....	72	35	26	19	69.6
Hawaiian soil 3128.....	114	56	35	32	52.6
Limonite.....	74	40	24	24	67.6
Goethite.....	36	29	25	21	77.8

all of these soils were of a similar nature. Samples of the same soils which had not been electrolyzed were subjected to a series of acid extractions, and the amounts of phosphate removed in the third extraction are given in table 6. In a general way these results are similar to those obtained after electrolysis.

*Nature of the phosphates formed when a soluble phosphate is added to soils and minerals.* It has been noted that when soluble phosphates are added to soils, varying quantities of the phosphate are converted into difficultly soluble forms. In order to determine whether compounds formed in this manner were similar to those present in soils after the soils had been electrolyzed, the following test was carried out. Half-gram samples of soil, which contained no readily soluble phosphates, were treated with 50 cc. of a solution containing 5 p.p.m. of phosphorus, and the suspension was allowed to go to dryness on the steam plate. This application is equivalent to adding 500 p.p.m. of phosphorus to

the soil. The soil was then subjected to a series of extractions with 0.002 N  $H_2SO_4$  at pH 3. The results of these extractions are given in table 7 and show that when phosphate is added to a soil a portion of it is converted into an insoluble form which seems to be similar in all the soils studied. They also show that the two hydrated iron oxides reacted in a similar manner. These results are in accordance with those reported by Ford (1).

Do any other compounds present in soils besides the hydrated iron compounds form difficultly soluble phosphates? Fraps (2) reported that various other soil minerals fix phosphorus. In order to obtain further information on this subject, a large variety of substances which were ground to pass a 100-mesh sieve, were

TABLE 8

*The amounts of phosphorus removed and not removed from various minerals and materials by extraction with 0.002 N  $H_2SO_4$  of pH 3, after treatment with 500 p.p.m. of phosphorus in the form of  $KH_2PO_4$*

MINERAL OR MATERIAL TREATED AND EXTRACTED	P.P.M. OF PHOSPHORUS REMOVED BY EXTRACTION WITH 0.002 N $H_2SO_4$				P.P.M. PHOSPHORUS NOT RECOVERED
	In first extraction	In second extraction	In third extraction	In fourth extraction	
Anorthoclase.....	700	160	48	8	0
Talc.....	530	0	.	.	0
Apophyllite.....	580	88	32	18	0
Nontronite.....	520	104	16	..	0
Red Granite.....	500	68	48	38	0
Kaolin.....	400	88	22	..	0
Muscovite.....	270	180	92	30	0
Biotite.....	288	112	56	15	29
Microcline.....	460	0	0	..	40
Orthoclase.....	420	20	0	..	60
Albite.....	425	0	0	..	75
Stilbite.....	412	0	0	..	88
Ca saturated Doucil.....	22	8	14	26	430
Meta Titanic Acid $TiO(OH)_2$ .....	0	0	0	..	500
Ortho Titanic Acid $Ti(OH)_3$ .....	0	0	0	..	500

treated with solutions of monobasic potassium phosphate ( $KH_2PO_4$ ) in the manner previously described. The results are given in table 8. These data indicate that there is a large variety of substances which have the ability to combine with phosphates, to a limited extent, forming difficultly soluble compounds. Of the substances listed, the titanic acids, which were prepared in this laboratory, and Doucil, which is a commercial zeolite used as a water softener, fixed phosphate to the greatest extent. In view of the fact that the presence of aluminosilicates of this type in soils is doubtful, it is believed that this latter class of substances would have little bearing on the phosphate fixation in soils. Generally speaking, soils contain only about 0.5 per cent of  $TiO_2$ , which is hardly sufficient to be of importance in comparison with the relatively large

amounts of hydrated iron oxides commonly present. However, data such as those presented in table 9 would suggest that in some soils titanium might be an important factor in phosphate fixation.

TABLE 9

*The total amount of titanium oxide in Hawaiian soils from different islands of the group\**

SOIL NUMBER	LOCATION	PER CENT $\text{TiO}_2$
626	Kauai	7.51
631	Maui	11.37
913	Hawaii	3.69
917	Oahu	3.53
920	Molokai	15.53

\* The writer is indebted to Dr. O. C. Magistad of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii, for data presented in this table.

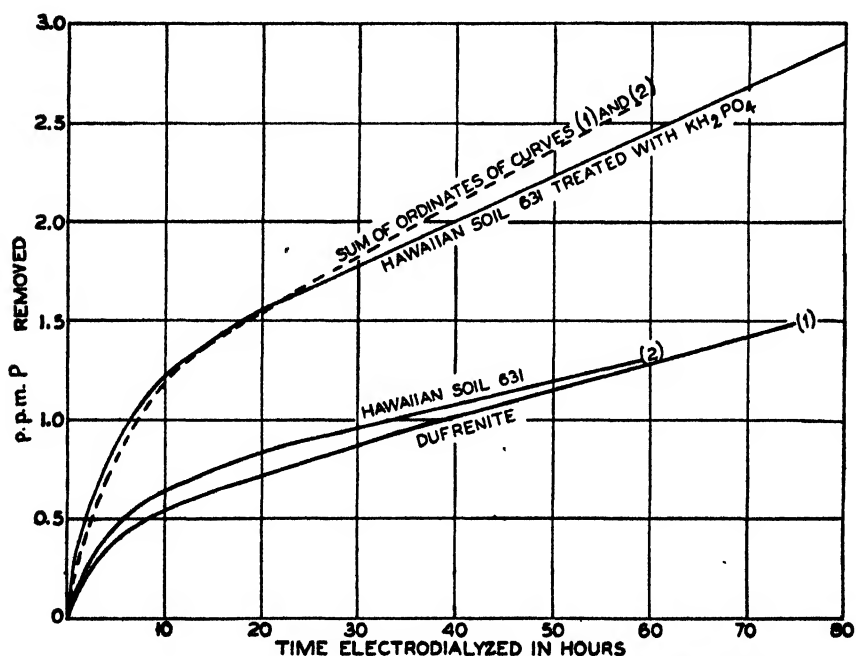


FIG. 6. RATE OF REMOVAL OF PHOSPHORUS BY ELECTRODIALYSIS FROM A TREATED AND AN UNTREATED SOIL AND FROM DUFRENITE. ALSO A CURVE BASED ON THE SUM OF THE CORRESPONDING ORDINATES OF THE TWO LOWER CURVES

In order to obtain further insight as to the nature of the difficultly soluble phosphates formed in soils, the rate of electrodialysis of a soil, which had received an application of phosphate, was studied. A 100-gm. sample of Hawaiian soil 631, 100 cc. of water, and 0.025 gm. of phosphorus in the form of

$\text{KH}_2\text{PO}_4$  were stirred together to form a suspension and then dried at  $60^\circ\text{C}$ . More water was added, and the suspension dried for a second time. The dried soil was then electrodialyzed in the usual manner. The results are shown in figure 6.

If the phosphorus added was converted into a compound similar to dufrenite, then the ordinates of the curve for the electrodialysis of 100 gm. of untreated Hawaiian soil 631, added to the corresponding ordinates of the curve for the electrodialysis of a sample of dufrenite containing 0.025 gm. of phosphorus, should be the same as the curve given for the electrodialysis of the soil sample to which 0.025 gm. of phosphorus had been added. Figure 6 shows that such is the case. It is, therefore, reasonable to infer that when a soluble phosphate is added to some soils, basic iron phosphates, similar to dufrenite, are formed. Ford (1) came to a similar conclusion.

#### SUMMARY

In a study of electrodialysis as a means of determining the nature of soil phosphates, the following points were covered: (a) the electrodialysis of readily soluble phosphate compounds; (b) the electrodialysis of difficultly soluble phosphate compounds; (c) the total quantity of phosphate removed by electrodialysis as compared to that removed by Truog's method of acid extraction; and (d) the nature of the difficultly soluble phosphates present in soils. The results of this study may be summarized as follows:

Either electrodialysis or extraction with acid removes readily soluble phosphate compounds from soils.

Electrodialysis does not remove phosphate from iron phosphates of soils nearly so rapidly as do extractions with  $0.002\text{ }N\text{ H}_2\text{SO}_4$ .

Electrodialysis for 60 hours removes more phosphate from some calcareous soils than do five extractions with  $0.002\text{ }N\text{ H}_2\text{SO}_4$ .

The difficultly soluble phosphate compounds of all the soils studied seemed to be similar, as revealed by deportment to electrodialysis and acid extractions.

The difficultly soluble phosphates formed by adding soluble phosphate to hydrated iron oxides appear to be similar to the difficultly soluble phosphates present in soils.

The phosphate naturally present in a lateritic soil containing 1.35 per cent phosphorus was similar in solubility to dufrenite.

The difficultly soluble phosphate compounds formed by the addition of soluble phosphate to some soils appears to be similar to dufrenite.

Under limited conditions, the specific rate of removal of phosphate from soils by electrodialysis may be used advantageously as an index of identification of the nature of the phosphate compounds present.

The determination of readily soluble phosphorus for practical purposes can be made not only more quickly and economically by acid extraction than by electrodialysis, but also with greater dependability.

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# THE FIXATION OF POTASH IN DIFFICULTLY AVAILABLE FORM IN SOILS<sup>1</sup>

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Numerous workers have shown that when soluble potash salts are applied to soils, the potassium is usually taken up quickly by the exchange material and held in exchangeable or readily available form, thus preventing serious loss in the drainage water (13). In time, a large portion of this exchangeable potassium is apparently transformed to a non-exchangeable and difficultly available form (5, 7, 22), the nature of which has, as yet, not been clearly elucidated.

Many years ago Dyer (5), at Rothamsted, worked with the soil of the Hoos Field, one plat of which had received 4,084 pounds per acre of potash over a period of 38 years. The potash removed by the crops and that remaining soluble in 1 per cent citric acid accounted for only about one-half of this amount, the remainder having either been leached away or converted into difficultly soluble forms. The latter explanation appears more plausible, since in five different groups of plats in the same field where potash plats and check plats could be compared, the treated plats invariably contained sufficient amounts of total potash to account for that applied.

About 25 years later Frear and Erb (7), working with the Pennsylvania Soil Fertility Plats, determined the potash content of a check plat and of one which had received 200 pounds per acre of potassium chloride biennially for 36 years (1881-1916), making in all a total of 1,793 pounds per acre of potash. Here again, as in the case of the Hoos Field, only about one-half of this potash was accounted for by that removed by the crops and remaining in the soil in readily available forms.

The present paper reports the results of an investigation of the nature of potash fixation in soils.

## EXPERIMENTAL

As already indicated, potash fixation may be of two kinds; namely, fixation as replaceable or readily available potash, and fixation as non-replaceable or

<sup>1</sup> Part of a thesis submitted to the faculty of the University of Wisconsin in partial fulfillment of the requirements for the degree of doctor of philosophy. This investigation was supported in part by a fellowship grant from the Agricultural and Scientific Bureau, N. Y. Potash Export My., Inc., New York, N. Y. Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

<sup>2</sup> The writer wishes to express his appreciation for the helpful suggestions and criticisms tendered by Prof. E. Truog and Prof. R. C. Emmons, under whose general direction this work was done.

difficultly available potash. In order to facilitate discussion, the expression "potash fixation" will be used in this paper in a restricted sense to include only the latter form of fixation.

In the experiments that follow, the readily available potash was extracted from the soil with a normal solution of ammonium acetate, and the analytical determinations of potassium were made by the permanganate titration of the cobalti-nitrite salt, as described elsewhere (30, 31).

TABLE 1

*Influence of wetting and drying soils in different ways on the fixation of potash added at the rate of 1,000 pounds per acre in the form of the chloride*

TEST NUMBER	TREATMENT OF THE SOILS AS REGARDS WETTING, DRYING, AND PERIOD OF INCUBATION	POUNDS PER ACRE OF POTASH FIXED	
		Miami silt loam	Hagerstown silt loam
1	Stood as water suspension for 5 minutes	0	0
2	Stood as water suspension for 1 day	0	0
3	Stood as water suspension for 5 days	0	0
4	Stood as water suspension for 10 days	0	0
5	Stood as wet paste for 5 minutes	0	0
6	Wet paste allowed to dry at room temperature 16 hours. Still very wet	0	0
7	Wet paste allowed to dry at room temperature 40 hours. Still moderately wet	0	0
8	Wet paste allowed to dry at room temperature 90 hours. Nearly dry	60	32
9	Wet paste allowed to dry at room temperature 125 hours. Completely air dry	300	252
10	Soil wetted and dried once at 70°C.	372	320
11	Soil wetted and dried 2 times at 70°C.	392	340
12	Soil wetted and dried 4 times at 70°C.	428	362
13	Soil wetted and dried 8 times at 70°C.	440	384
14	Soil wetted and dried 16 times at 70°C.	464	396
15	Soil wetted and dried 24 times at 70°C.	476	424
16	Soil wetted and dried 32 times at 70°C.	488	428

### *Conditions favoring potash fixation in soils*

Preliminary tests revealed that potassium in the form of soluble salts is not fixed by soils to a great extent from dilute solutions unless drying of the soil takes place. Tests were therefore undertaken to show the influence of moisture supply and wetting and drying of the soil on the fixation of potassium.

Two soils, Miami silt loam and Hagerstown silt loam, were used for the study. Twenty-five-gram portions of soil were placed in 500-cc. Erlenmeyer flasks, to each of which was added 35 cc. of water containing sufficient potassium chloride to equal an application of 1,000 pounds per acre of potash. In some cases the available potash of the soils in the flasks was immediately extracted with

500 cc. of normal ammonium acetate; in other cases the soils with added water were allowed to stand for several days; in others they were allowed to dry slowly; and in still others they were alternately wetted and dried at 70°C. several times before being extracted with normal ammonium acetate. The details of the treatments and the results are given in table 1. It is evident that little or no potash fixation occurred until drying began to take place, and that repeated wetting and drying increased the amount of fixation. For the two soils studied, approximately 75 per cent of the potash fixation took place with one drying at 70°C. as compared with that after 32 wettings and dryings at 70°C.

TABLE 2

*Influence of continuous moistness versus alternate wetting and drying on the amount of potash fixed by a Miami silt loam treated with 10 tons per acre of potash as the bicarbonate*

The soil contained 2.14 per cent of total potash before treatment

NUM- BER OF TEST	TREATMENT OF SOIL AFTER ADDITION OF POTASSIUM BICARBONATE	PERCENTAGE OF TOTAL POTASH IN THE SOIL AFTER REMOVAL OF ALL THAT IS SOLUBLE AND RE- PLACEABLE	POUNDS PER ACRE OF POTASH FIXED BY THE SOIL	PERCENTAGE OF ADDED POTASH FIXED
1	Soil-water ratio made 1 to 3 and stood for 3 days	2.14	0	0
2	Optimum moisture conditions for 3 days	2.13	0	0
3	Soil moistened and allowed to dry slowly at room temperature for 3 days	2.20	1,200	6
4	Soil moistened and dried at once at 60°C.	2.32	3,600	18
5	Soil alternately wetted and dried 3 times at 60°C.	2.38	4,980	25
6	Soil alternately wetted and dried 6 times at 60°C.	2.40	5,388	27

To test further whether or not potash fixation would occur under moist conditions, 30-gm. portions of Miami silt loam and of Hagerstown silt loam were placed in carbon filter funnels and leached at the rate of 2 cc. per hour for 60 consecutive days with a solution containing 40 p.p.m. of potassium as the bicarbonate. Subsequent analyses showed that no appreciable amount of potassium had been fixed in a difficultly soluble form.

Since the effect of drying may be only that of concentration of the potassium salts, and thus subsequently due to mass action, more of the potassium is caused to go into difficultly available forms, a second set of tests was conducted to determine whether or not fixation would occur from concentrated solutions without the drying process. Miami silt loam was treated with potash in the form of the bicarbonate at the rate of 10 tons per acre. Portions of the treated and moistened soil were placed in test tubes and subjected to the various wet-



ting and drying treatments described in table 2. After treatment, the soils in the test tubes were completely freed of soluble and replaceable potash and analyzed for the total potash remaining in the soil. The differences in total potash between the treated and untreated portions of the soil give the amounts fixed. These results (table 2) substantiate those of the first set of tests; i.e., little or no fixation occurs until drying takes place.

There appeared to be a remote possibility that the potassium salts are held mechanically as inclusions in the partially dehydrated colloids of the soil, and

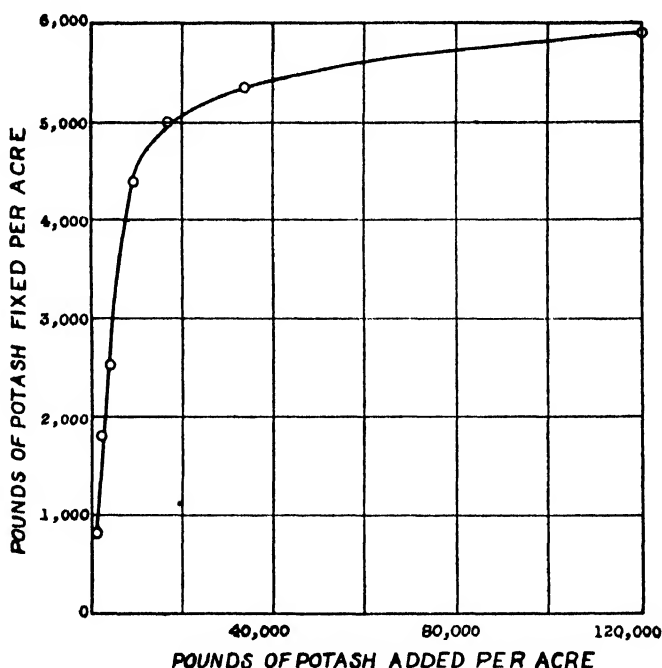


FIG. 1. EFFECT OF THE CONCENTRATION OF SOLUBLE POTASH ON THE AMOUNT FIXED BY MIAMI SILT LOAM WHEN TREATED WITH VARYING QUANTITIES OF POTASSIUM BICARBONATE AND THEN ALTERNATELY WETTED AND DRIED 10 TIMES AT 70°C.

that the potash is not actually fixed chemically. To test this, a synthetic soil was made, using the proper proportions of different sizes of quartz sand, together with silica gel so as to fit the results of a mechanical analysis of Miami silt loam. To this synthetic soil was added 10 tons per acre of potash as the bicarbonate, and the whole wetted and dried 10 times at 70°C. The dried material was found to retain no appreciable amount of potash.

Thus it appears that drying causes the formation of a potash-containing secondary mineral from which the potash is not brought into solution by water or ammonium acetate solution. Further evidence substantiating this theory will be given later.

*Effect of the concentration of soluble potassium on fixation*

In order to test the effect of the concentration of soluble potassium on the rate of fixation, eight 20-gm. portions of Miami silt loam were placed in test tubes and treated with 0, 1,000, 2,000, 4,000, 8,000, 16,000, 32,000, and 120,000 pounds per acre of potash as the bicarbonate respectively. The contents of each test tube were then wetted and dried 10 times at 70°C. The samples were

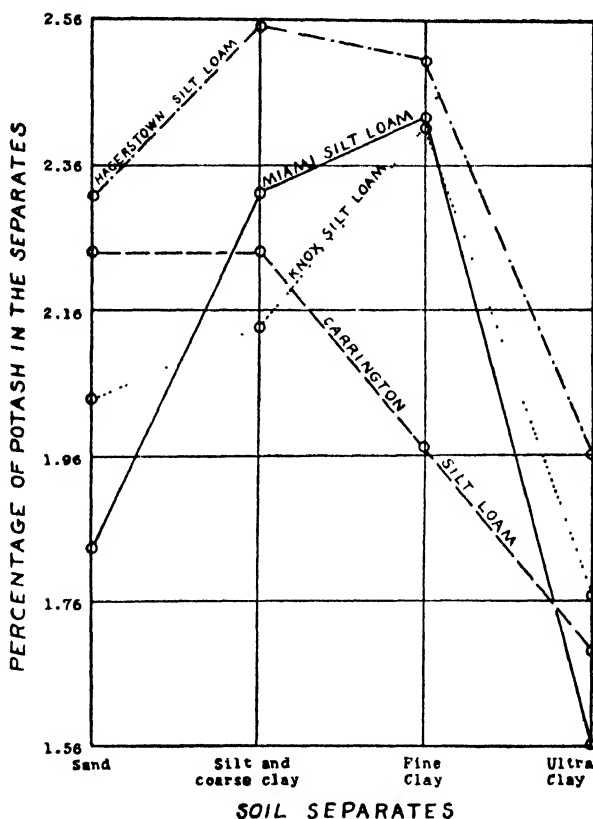


FIG. 2. POTASH CONTENT OF THE TEXTURAL SEPARATES OF FOUR SOILS

next leached with normal ammonium acetate to free them of readily available potassium, and then analyzed for total potash. The results of the analyses, given in graphical form in figure 1, show that the amount of potash fixed increased rapidly from an application of 1,000 pounds to 16,000 pounds, and that beyond an application of 32,000 pounds, very little increase in fixation took place. Approximately 80 per cent of the 1,000-pound application was fixed, and only 17 per cent of the 32,000-pound application. Thus, it appears that under the conditions of the experiment a soil will fix a maximum of potash in a very short time.

*Soil separates as regards total potash and fixation*

Several workers have shown that the potash content of a soil is not constant for its different mechanical separates. Soils investigated by Dumont (3) contained, in one case, five-sixths of the potash in the fine material, and one-sixth in the sands; and in another case, three-tenths in the fine material, and seven-tenths in the coarse. Steinkoenig (28) found that the potash content increased with fineness, and Gericke (9) found it concentrated in the particles ranging from 0.02 mm. to 0.002 mm. in diameter.

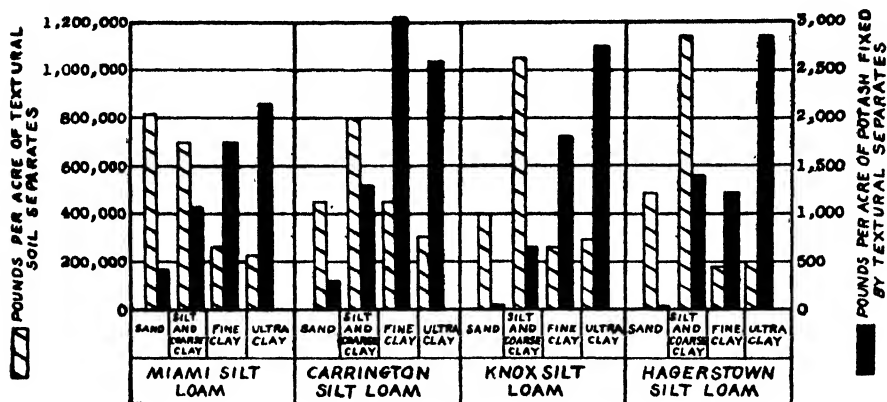


FIG. 3. AMOUNTS OF POTASH PER ACRE FIXED IN DIFFICULTLY AVAILABLE FORM BY THE TEXTURAL SEPARATES OF FOUR SOILS TREATED WITH 60 TONS PER ACRE OF POTASH AND ALTERNATELY WETTED AND DRIED 10 TIMES AT 70°C.

Four soils were simultaneously separated into four textural separates by centrifuging, and the resultant separates analyzed for total potash. The approximate diameters of the particles in the separates were:

- Sands..... >0.05 mm.
- Silt and coarse clay..... <0.05 mm. and >0.002 mm.
- Fine clay..... <0.002 mm. and >0.0003 mm.
- Ultra clay..... <0.0003 mm.

The data presented graphically in figure 2 show that for the four soils studied, the ultra clay separates have the lowest percentage of total potash, the sands the next lowest percentage, and the intermediate separates have the highest. These results suggest that the sand separates contain the greatest percentage of primary quartz, the medium sized separates contain the greatest percentage of feldspars, and the ultra clay consists mainly of secondary minerals containing less potash as a whole. The natural question arising at this point is: What separate exercises the greatest fixing power for potash?

Using the same four soils, another experiment was conducted in which two 40-gm. portions of each soil were placed in beakers. One of each pair was treated with 60 tons per acre of potash as the bicarbonate, and all were wetted

and dried 10 times at 70°C. The soils, treated and untreated, were then dispersed and leached as free as possible of soluble and replaceable potash by normal ammonium acetate, placed in centrifuge tubes and separated into four textural separates as previously described. Results of analyses for total potash are shown graphically in figure 3. In every case it will be noted that the ultra-clay is the highest fixer, fine clay next, and so on to the sand, where practically no fixation took place. The slight fixation by the coarse separates is attributed to the colloidal material that adhered to the surface of the grains and could be easily seen with a microscope. By taking a small portion of this coarse material and removing the colloidal material by rubbing, it was possible to reduce the treated and untreated coarse separates to nearly identical percentages of total potash. The specific fixing power of the soil separates of the four soils was calculated as follows:  $\frac{\text{Weight of potash fixed}}{\text{Weight of separate}} \times 10,000 = \text{specific fixing power}$ .

These calculations gave the results that follow: sand, 0 to 6; silt and coarse clay, 6 to 16; fine clay, 66 to 69; ultra clay, 82 to 140.

#### *Capacity of various soils to fix added potash*

Before attempting to determine the identity of the substances in soils causing potash fixation, a large number of soils were studied for their capacity to fix potash. One hundred samples, obtained from various parts of the United States, Hawaii, and Central America, were studied. Samples of the soils were placed in 500-cc. Erlenmeyer flasks, moistened with a solution of potassium chloride equivalent to 1,000 pounds per acre of potash, and immediately extracted with normal ammonium acetate. The potash was determined in the extract. A second set of samples, similarly treated with potash, were wetted and dried alternately 10 times on a hot plate at 70°C. and then extracted with normal ammonium acetate as before. The difference between the potash content of the extracts of the two sets of tests gives the amounts of potash fixed by the soils. The results given in table 3 show that fixation varies from 0 to 114 per cent of the 1,000 pounds added. The seven soils which ran over 100 per cent apparently fixed not only the 1000 pounds added, but some of the available potash originally present in them.

As a rule, the lower the colloid content of the soil, the lower the fixing capacity, and the higher the colloid content, the higher the fixing capacity. However, there are some exceptions—samples 39 and 40 are Hawaiian lateritic soils containing 90 per cent or more of colloidal material, yet they fix no potash. Other exceptions can be found by examining table 2. Thus it is evident that the quality of the colloid of a soil, as well as the quantity, is important in determining its fixing capacity for potassium

#### *Effect of continued potash fertilization on the residual capacity of a soil to fix potash*

Two samples of Hagerstown silt loam were obtained, one of which had received no potash fertilizer, and the other, 5,000 pounds of potassium chloride

TABLE 3

*Amounts of potash fixed by 100 different soils after potash was added at the rate of 1,000 pounds per acre and then alternately wetted and dried 10 times at 70°C.*

NO.	SOURCE OF SAMPLE	TYPE OR KIND OF SOIL	DEPTH OF SAMPLE	AMOUNTS OF POTASH FIXED BY THE SOILS		
				Pounds per acre		Per cent of addition, average
				a	b	
39	Hawaii	Laterite No. 5	Surface	0	0	0
40	Hawaii	Laterite No. 10	Surface	0	0	0
20	Columbia Co., Wis.	Dunning F. S. L.	Surface	0	0	0
22	Washburn Co., Wis.	Vilas sand	Surface	0	0	0
24	Sauk Co., Wis.	Plainfield F. S.	Surface	0	0	0
25	Washburn Co., Wis.	Plainfield sand	Surface	0	0	0
32	Cape Girardeau, Mo.	Kaolin	...	0	0	0
47	Sparta, Wis.	Plainfield sand	Surface	0	0	0
48	Hancock, Wis.	Plainfield sand	Surface	0	0	0
78	Fresno Co., Calif.	Fresno sand	Surface	0	0	0
69	Montgomery Co., Kas.	Crawford loam	Surface	0	0	0
70	Montgomery Co., Kas.	Crawford loam	Subsoil	809	809	81*
13	Iowa Co., Wis.	Dodgeville Si. L.	Surface	33	23	3
23	Ashland Co., Wis.	Vilas sand	Surface	50	30	4
17	Ashland Co., Wis.	Mellen loam	Surface	60	65	6
26	Waushara Co., Wis.	Coloma sand	Surface	76	...	8
79	New Iberia, La.	Peat	Surface	100	100	10
43	Wisconsin	Knox Si. L.	Surface	121	81	10
27	Columbia Co., Wis.	Coloma sand	Surface	119	.	12
11	Iowa Co., Wis.	Knox Si. L.	Surface	125	125	13
12	Iowa Co., Wis.	Knox Si. L.	Subsoil	338	338	34*
7	Dane Co., Wis.	Miami Si. L.	Surface	131	140	14
8	Dane Co., Wis.	Miami Si. L.	Subsoil	848	848	85*
19	Columbia Co., Wis.	Miami F. S. L.	Surface	144	144	14
46	Marshfield, Wis.	Colby Si. L.	Surface	118	183	15
67	New Iberia, La.	Oliver loam	Surface	142	172	16
68	New Iberia, La.	Oliver loam	Subsoil	186	216	20*
45	Wisconsin	Carrington Si. L.	Surface	163	163	16
28	Dane Co., Wis.	Peat	Surface	146	180	16
73	California	Hanford F. S. L.	Surface	173	173	17
76	California	San Joaquin loam	Surface	190	180	18
18	Douglas Co., Wis.	Mellen loam	Surface	185	195	19
58	California	Imperial clay	Surface	230	180	20
41	Hawaii	Laterite No. 9	Surface	205	215	21
65	New York State	Dutchess stony Si. L.	Surface	229	209	22
38	Ashland, Wis.	Superior clay	Surface	250	205	23
30	Penn. Potash Plat†	Hagerstown Si. L.	Surface	255	230	24

\* Subsoil sample corresponding to the surface sample reported immediately below in the table.

† The plat from which this sample was taken received 5,000 pounds of potash over a period of 50 years.

TABLE 3—Continued

NO	SOURCE OF SAMPLE	TYPE OR KIND OF SOIL	DEPTH OF SAMPLE	AMOUNTS OF POTASH FIXED BY THE SOILS		
				Pounds per acre		Per cent of addition, average
				a	b	
37	Wisconsin	Boone S. L.	Surface	273	251	26
63	New Iberia, La.	Iberia Si. L.	Surface	272	262	27
64	New Iberia, La.	Iberia Si. L.	Subsoil	536	536	54*
44	Wisconsin	Carrington Si. L.	Surface	261	282	27
61	Montgomery Co., Kas.	Oswego Si. L.	Surface	305	305	30
9	Fond du Lac Co., Wis.	Fox Si. L.	Surface	308	308	31
10	Fond du Lac Co., Wis.	Fox Si. L.	Subsoil	392	338	36*
14	Dane Co., Wis.	Clyde Si. L.	Surface	285	350	32
3	Fond du Lac Co., Wis.	Superior clay	Surface	315	328	32
4	Fond du Lac Co., Wis.	Superior clay	Subsoil	696	686	69*
54	California	Merced clay adobe	Surface	285	390	34
96	Honduras, C. A.	Alluvial lowland	Surface	333	358	35
21	Waushara Co., Wis.	Coloma S. L.	Surface	360	350	35
50	Wisconsin	Dodgeville Si. L.	Surface	370	370	37
77	California	San Joaquin S. L.	Surface	370	370	37
81	Honduras, C. A.	Alluvial lowland	Surface	382	390	39
74	Calhoun, Ia.	Orangeburg S. L.	Surface	401	401	40
75	Calhoun, Ia.	Orangeburg S. L.	Subsoil	392	382	39*
42	Wisconsin	Knox Si. L.	Surface	410	...	41
29	Penn. Check Plat	Hagerstown Si. L.	Surface	405	425	41
99	Honduras, C. A.	Alluvial lowland	Surface	425	415	42
57	California	Porterville clay adobe	Surface	365	495	43
95	Honduras, C. A.	Alluvial lowland	Surface	435	455	44
31	Dane Co., Wis.	Miami Si. L.	Surface	444	454	45
49	Wisconsin	Dodgeville Si. L.	Surface	483	503	49
51	Wisconsin	Waukesha Si. L.	Surface	525	555	54
71	New Iberia, La.	Miller loam	Surface	543	543	54
72	New Iberia, La.	Miller loam	Subsoil	785	765	77*
52	Atlanta, Ga.	Weathered Gneiss	Surface	559	537	55
53	Atlanta, Ga.	Weathered Gneiss	Subsoil	116	116	12*
15	Fond du Lac Co., Wis.	Clyde C. L.	Surface	566	534	55
16	Fond du Lac Co., Wis.	Clyde C. L.	Subsoil	850	893	87*
90	Honduras, C. A.	Alluvial lowland	Surface	565	585	57
62	Baton Rouge, La.	Memphis Si. L.	Surface	588	588	59
80	Honduras, C. A.	Alluvial lowland	Surface	600	580	59
1	Ashland Co., Wis.	Superior clay	Surface	603	603	60
2	Ashland Co., Wis.	Superior clay	Subsoil	763	785	77*
94	Honduras, C. A.	Alluvial lowland	Surface	605	620	61
82	Honduras, C. A.	Alluvial lowland	Surface	645	605	62
5	Fond du Lac Co., Wis.	Poygan clay	Surface	665	643	65
6	Fond du Lac Co., Wis.	Poygan clay	Subsoil	719	708	71*
55	New Iberia, La.	Oliver clay	Surface	675	635	65
56	New Iberia, La.	Oliver clay	Subsoil	415	425	42*
66	New Iberia, La.	Iberia C. L.	Surface	688	688	69

TABLE 3—*Concluded*

NO.	SOURCE OF SAMPLE	TYPE OR KIND OF SOIL	DEPTH OF SAMPLE	AMOUNTS OF POTASH FIXED BY THE SOILS		
				Pounds per acre		Per cent of addition, average
				a	b	
77	Honduras, C. A.	Alluvial lowland	Surface	750	780	76
100	Honduras, C. A.	Alluvial lowland	Surface	807	810	81
91	Honduras, C. A.	Alluvial lowland	Surface	818	808	81
93	Honduras, C. A.	Alluvial lowland	Surface	806	828	82
59	Montgomery Co., Kas.	Summit Si. C. L.	Surface	840	830	83
60	Montgomery Co., Kas.	Summit Si. C. L.	Subsoil	700	700	70*
89	Honduras, C. A.	Alluvial lowland	Surface	841	884	86
98	Honduras, C. A.	Alluvial lowland	Surface	862	873	87
87	Honduras, C. A.	Alluvial lowland	Surface	1,033	1,010	102
84	Honduras, C. A.	Alluvial lowland	Surface	1,033	1,033	103
92	Honduras, C. A.	Alluvial lowland	Surface	1,057	1,024	104
86	Honduras, C. A.	Alluvial lowland	Surface	1,061	1,061	106
88	Honduras, C. A.	Alluvial lowland	Surface	1,093	1,083	108
83	Honduras, C. A.	Alluvial lowland	Surface	1,088	1,088	109
85	Honduras, C. A.	Alluvial lowland	Surface	1,122	1,154	114

over a period of 50 years. Both samples were treated for their capacity to fix potash, and the results are given in table 4. It will be noted that the soil which had received the potash fertilizer in the field fixed only about half as much potash as the unfertilized soil. This would lead one to believe that this soil had fixed a considerable portion of the potash applied, and thus a portion of its fixing material had become saturated.

#### *Effect of lime on fixation*

A few isolated tests indicated that liming may be a factor in potash fixation. To ascertain more definitely its effect, experiments were conducted on samples of several soils which were given three distinct treatments; viz., (a) one portion of each soil was alternately wetted and dried 10 times at 70°C. (b) a second portion was treated with 1,000 pounds per acre of potash as the chloride and then alternately wetted and dried 10 times at 70°C. and (c) a third portion was treated with 3 tons per acre of calcium hydroxide as lime water, 1,000 pounds per acre of potash as the chloride, and then alternately wetted and dried 10 times at 70°C. All portions of the soils were then dispersed in normal ammonium acetate, thrown on a filter, and leached practically free of all replaceable and soluble potash. The potash in the leachate was determined in the usual manner.

The results given in table 5 show quite conclusively that lime additions do cause increased potash fixation in soils. The reason for this increase has not been ascertained as yet, but it may be due to the fact that the alkaline condi-

tion produced by the lime hydroxide brings alumina and silica into solution, which, in turn, combine with the potash to form an insoluble potassium aluminum silicate. This increased fixation, caused by lime additions, may explain in part the results obtained by Bledsoe (2), Goethe (8), McCall (16), Plummer (23), and others, who found that calcium carbonate additions in some way limit the amount of potash which the plant is able to take up. Also, it would explain the fact that Lyon and Bizzell (14, 15), MacIntire et al. (18, 19, 20), McCool (17), and Spurway (24) found that calcium carbonate additions do not increase the potash content of drainage water.

#### *Potash fixation by soil minerals*

To find out whether a definite mineral in the soil causes the fixation of potash, a series of minerals were finely ground, treated with potassium chloride, and alternately wetted and dried 10 times at 70°C. The results indicated that biotite, nephelite, syenite, anorthite, apophyllite, anorthoclase, chlorite, and

TABLE 4

*Effect of 50 years of potash fertilization on Hagerstown silt loam, Pennsylvania station plats, on the potash fixing power of the soil*

PLAT TREATMENT IN FIELD	AMOUNTS OF POTASH PER ACRE FIXED FROM A 1,000-POUND LABORATORY APPLICATION		
	Pounds per acre		Per cent of addition
	Test 1	Test 2	
No potash	405	425	41
5,000 pounds of potash added over a 50-year period. . . . .	255	230	24

talc do not fix potash; but that albite, halloysite, deweylite, and decomposed granite do fix small quantities, though not enough to account for the large fixation in soils.

Minerals isolated by specific gravity methods (29) from soil possessing a fairly high fixing power were treated with 10 tons per acre of potash as the bicarbonate, and were alternately wetted and dried 10 times at 70°C. to induce fixation. Only that portion of the soil having particles >0.002 mm. in diameter was separated into its mineral constituents. The remainder of the soil, the colloidal fraction, was tested as a unit for fixation. The results given in table 6 indicate that very little fixation took place in any of the soil minerals of a diameter greater than 0.002 mm. except for the fraction lighter than specific gravity 2.500, which contained a large amount of undispersed aggregates of colloidal matter. On the other hand, the colloidal fraction of this soil (diameter <0.002 mm.) showed an increase of 60 per cent in total potash content, due to fixation. Thus, it is evident that one should study the colloidal fraction of a soil if one wishes to investigate the constituents causing potash fixation.



TABLE 5

*Amounts of potash fixed by limed and unlimed soils*

Soils were treated with 1,000 pounds per acre of potash as the chloride and wetted and dried 10 times at 70°C.

SOIL CLASSIFICATION	POUNDS OF POTASH FIXED PER ACRE		
	Unlimed	Limed with 3 tons per acre of calcium hydroxide	Increase due to lime
Hagerstown silt loam .....	410	488	78
Laterite No. 9. ....	210	287	77
Dodgeville silt loam. ....	370	422	52
Carrington silt loam. ....	270	353	83
Knox silt loam .....	410	442	32
Waukesha silt loam. ....	540	559	19
Colby silt loam .....	150	150	0
Superior clay. ....	600	664	64
Carrington silt loam. ....	160	228	68
Miami silt loam. ....	450	450	0
Knox silt loam. ....	100	100	0

TABLE 6

*Amounts of potash fixed by minerals from Hagerstown silt loam (Pennsylvania station), when treated at the rate of 10 tons per acre of potash as the bicarbonate, and then wetted and dried 10 times at 60°C.*

DIAMETER OF THE MINERAL PARTICLES	SPECIFIC GRAVITY RANGE OF THE MINERAL FRACTIONS	IMPORTANT MINERALS FOUND IN EACH SPECIFIC GRAVITY RANGE OR FRACTION	AMOUNT OF EACH FRACTION CONTAINED IN ONE ACRE OF SOIL	TOTAL NON-REPLACEABLE POTASH IN EACH FRACTION		POTASH FIXATION DURING TREATMENT	
				Before treatment with potassium bicarbonate	After treatment with potassium bicarbonate	As percentage of original total	In pounds per acre
<i>mm.</i>			<i>pounds</i>	<i>per cent</i>	<i>per cent</i>		
> 0.002	> 2.750	Mica plus heavy iron minerals	36,501	1.20	1.34	11.7	44
	< 2.750	Plagioclase, mica, and some quartz	36,249	1.50	1.54	2.8	14
	> 2.655	Mainly quartz	664,091	0.78	0.78	0.0	0
	< 2.655						
	> 2.620						
	< 2.620	Mainly quartz and feldspars	315,733	2.00	2.05	2.5	158
	> 2.600						
	< 2.600	Mainly feldspars	270,300	7.98	8.08	1.3	270
	> 2.500						
	< 2.500	Feldspars plus a large amount of low specific gravity minerals	38,674	1.98	2.39	20.7	158
< 0.002 (Colloid)	Not determined	No specific gravity separations made	461,797	1.88	3.01	60.1	5,218

*Influence of leaching soils with HCl and Na<sub>2</sub>CO<sub>3</sub> solutions on potash fixation*

It was thought that if one leached a soil with HCl or Na<sub>2</sub>CO<sub>3</sub> solutions one might be able to destroy its fixing capacity by removing the fixing material. Two soils were selected, Miami silt loam and Hagerstown silt loam. Twenty-five-gram samples of each were leached 132 hours with *N* HCl, 5,500 cc. being used. Also, a 25-gm. sample of each was leached with *N* Na<sub>2</sub>CO<sub>3</sub> for 240 hours, using 5,000 cc. of solution. The leachates were analyzed for alumina and silica, and the leached soils, after being thoroughly washed with water, were tested for fixation of potash. The results, given in table 7, show that the HCl leaching decreased the fixing capacity, whereas the Na<sub>2</sub>CO<sub>3</sub> treatment increased it. The analyses of the leachates indicate that aluminum may be in some manner tied up with the fixation of potash. In the case of the HCl treatment, a large amount of Al<sub>2</sub>O<sub>3</sub> was removed, and fixation dropped; where-

TABLE 7

*Influence of removal of Al<sub>2</sub>O<sub>3</sub> and SiO<sub>2</sub> from soils by leaching with *N* Na<sub>2</sub>CO<sub>3</sub> and *N* HCl on potash fixation*

CLASS OF SOIL	PERCENTAGE OF APPLIED POTASH FIXED BY SOILS INDICATED			AMOUNTS REMOVED FROM 25-GM. SAMPLES		
	Normal field soil	Soil leached with <i>N</i> HCl	Soil leached with <i>N</i> Na <sub>2</sub> CO <sub>3</sub>	By HCl leaching	By Na <sub>2</sub> CO <sub>3</sub> leaching	
				Al <sub>2</sub> O <sub>3</sub>	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>
				gm.	gm.	gm.
Miami silt loam. . . . .	45	21	56	1 639	0.125	0 339
Hagerstown silt loam. . . . .	41	28	75	1.531	0.237	0.177

as in the case of the Na<sub>2</sub>CO<sub>3</sub> treatment, only about one-fifth as much Al<sub>2</sub>O<sub>3</sub> was removed, and the fixation was increased, a condition which could be explained on the assumption that the Na<sub>2</sub>CO<sub>3</sub> formed sodium aluminate, which was retained in the soil and aided potash fixation.

*Fixation of potash by synthetic mixtures of sand, silica gel, alumina gel, and calcium hydroxide*

To test further the possibility of potash being fixed by its mutual action with silica gel and alumina gel, a series of tests was conducted as tabulated in table 8. Various proportions of silica gel, alumina gel, calcium hydroxide, potassium chloride, and pure sand were mixed together. These mixtures were then wetted and dried alternately 10 times at 70°C. to induce fixation. However, no fixation took place, even under alkaline conditions, since all the potash added was removed by extraction with normal ammonium acetate.

Before further studies were undertaken in an attempt to determine the compounds in the soil responsible for potash fixation, an attempt was made to isolate the compound formed during potash fixation.

*Form of potash fixation in Hagerstown silt loam*

From the results reported thus far, it is evident that a portion of the soluble potassium added to a soil becomes fixed in a difficultly available form. Evidence also points to the fact that it is not held mechanically. Thus, it seems probable that it is fixed in the form of a new secondary mineral of extremely low solubility. In order to isolate this new mineral, a sample of soil was needed which had been treated for a number of years with potassium salts. For this purpose, samples of Hagerstown silt loam were obtained from plats 1 and 4 of the Pennsylvania station<sup>3</sup> soil fertility field started 50 years ago. Plat 1 is a check and plat 4 has received 200 pounds per acre of potassium chloride biennially since 1881, making 3,158 pounds of potash in all. Approximately 600

TABLE 8

*Fixation of potash by various synthetic mixtures. All mixtures were wetted and then dried at 70°C., 10 times*

TEST NUMBER	COMPOSITION OF EACH TEST MIXTURE					pH OF THE TEST MIXTURE AT THE END OF THE WET- TING AND DRYING PERIOD	EXTENT OF POTASH FIXATION
	Medium white sand	H <sub>2</sub> SiO <sub>3</sub> gel	Al(OH) <sub>3</sub> gel	Ca(OH) <sub>2</sub>	KCl		
	gm.	gm.	gm.	gm.	gm.		
1	10	0.5	.....	.....	0.008	4.0	None
2	10	0.5	.....	0.25	0.008	8.5	None
3	10	.....	0.5	.....	0.008	8.5	None
4	10	.....	0.5	0.25	0.008	8.5	None
5	10	0.25	0.25	.....	0.008	6.0	None
6	10	0.25	0.25	0.25	0.008	8.5	None

pounds of potash have been removed from the potassium chloride plat in excess of that removed from the check plat by the crops corn, oats, wheat, and hay (7). Also, the sample of soil from the potassium chloride plat contains an excess over the check plat of 336 pounds of available potash. This leaves 2,222 pounds of potash unaccounted for, the greater portion of which is probably fixed in the soil, since potash leaches but little (13).

Preliminary attempts at separating the soil minerals by specific gravity methods led to many difficulties, and methods of separation had to be developed to fit the needs. These methods have been reported elsewhere in detail (29).

Using the methods described elsewhere (29, 30, 31), mechanical, mineralogical, and chemical analyses were made of the Hagerstown silt loam of check plat 1 and potassium chloride plat 4. As shown in table 9, the potassium chloride plat contains 9,266 pounds of total potash in excess of that contained

<sup>3</sup> The writer is greatly indebted to the officials of the Pennsylvania Agricultural Experiment Station for having furnished the samples of Hagerstown silt loam used in this investigation.

TABLE 9

*Amounts of minerals and total potash in the soil separates and fractions thereof of the treated and untreated plots of Hagerstown silt loam, Pennsylvania station, the potassium chloride plat having received the equivalent of 3,158 pounds of potash over a period of 50 years, and the check plat having received none*

PART OF SAMPLE	SPECIFIC GRAVITY RANGE OF EACH FRACTION PRESENT	MAIN MINERALS PRESENT IN EACH FRACTION	POUNDS OF SEPARATES AND MINERALS IN AN ACRE OF SOIL		PERCENT-AGE TOTAL POTASH IN EACH SEPARATE OR FRACTION		POUNDS OF POTASH PER ACRE IN THE SEPARATES AND FRACTIONS		POUNDS PER ACRE OF EXCESS POTASH IN KCl PLAT OVER THAT IN THE CHECK PLAT
			KCl Plat	Check Plat	KCl Plat	Check Plat	KCl Plat	Check Plat	
Entire sample	Not determined	Not determined	1,873,385	1,893,401	2.90	2.38	54,328	45,062	+9,266
Coarse and separate	Not determined	Not determined	52,455	70,056	0.62	0.72	325	504	-179
40 mesh to 0.002 mm. separate	>2.750	Mica plus heavy iron minerals	32,420	36,501	1.28	1.20	415	439	-24
	<2.750 >2.655	Plagioclase, mica and some quartz	28,440	36,249	1.61	1.50	458	544	-86
	<2.655 >2.620	Nearly pure quartz plus some feldspar	614,731	664,091	0.79	0.79	4,854	5,246	-392
	<2.620 >2.600	Mixtures of feldspar and quartz	222,392	315,733	2.11	1.99	4,692	6,283	-1,591
	<2.600 >2.500	Nearly all feldspar	366,279	270,300	8.11	7.99	29,705	21,597	+8,108
	<2.500	Some feldspar plus the light minerals	84,746	38,674	2.04	1.98	1,729	766	+963
	<0.002 mm. separate	Mainly hydrated secondary minerals	472,122	461,797	2.46	1.88	11,614	8,682	+2,932
Sum of separates			1,873,385	1,893,401	...	...	53,792	44,061	+9,731

in the check plat, but only 2,558 pounds of this amount could have come from the potash added as fertilizer. Since the potassium chloride plat contains considerably more feldspar than the check plat, it is believed that this excess potassium exists as native feldspar. Undoubtedly this difference in the amount of feldspar existed at the time the plats were laid out.

The tests previously discussed in this paper show that potassium fixation occurs almost entirely in the colloidal fraction of the soil. In the colloidal separate ( $<0.002$  mm. diameter), table 9, the total potash content is 2.46 per cent for the potassium chloride plat, and 1.88 per cent for the check plat, or an increase of 0.58 per cent in the potassium chloride plat. Thus, it was

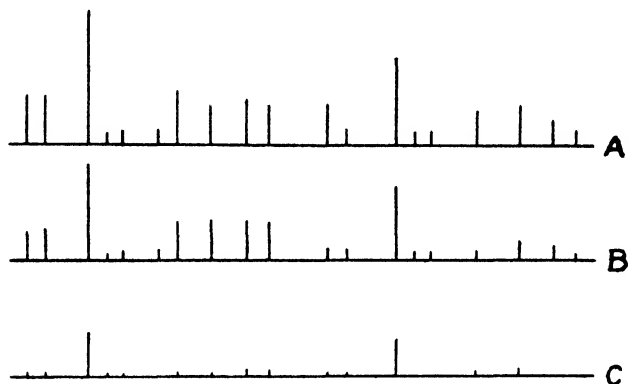


FIG. 4. A COMPARISON OF THE X-RAY PATTERN OF PURE MUSCOVITE WITH THE PATTERNS OF THE HEAVY COLLOIDAL FRACTIONS OF THE POTASSIUM CHLORIDE AND CHECK PLATS OF HAGERSTOWN SILT LOAM, SHOWING MUSCOVITE TO BE MORE PLENTIFUL IN THE POTASSIUM CHLORIDE PLAT

A—Pure muscovite.

B—Potassium chloride plat. Diameter of particles 0.002 to 0.0003 mm. Specific gravity of particles 2.667.

C—Check plat. Diameter of particles 0.002 to 0.0003 mm. Specific gravity of particles 2.667.

decided to study the colloidal fractions of these soils further. Since particles below 0.0003 mm. in diameter could not be separated into mineral fractions by specific gravity means, a separate consisting of particles 0.002 to 0.0003 mm. in diameter was mechanically isolated and separated at specific gravity 2.667. In this manner, all the silica, feldspar, and lighter minerals were separated from the heavier ones. Since petrographic methods (6, 32) could not be applied to these fine fractions as a means of identifying the minerals present, X-ray patterns were obtained for this purpose. The lighter mineral fractions showed only quartz lines, whereas the heavier fractions showed muscovite lines. In the case of the potassium chloride plat, the muscovite lines extended through the second order quite completely, whereas in the case of the check plat they extended into the second order only faintly and incompletely (fig. 4).

This is an indication of the relative amounts of muscovite in the two samples, since their X-ray analyses were made simultaneously.

Chemical analyses were also made of these mineral fractions, as given in table 10. Since the potash content of the coarse portion of the colloid separate ( $<0.002$  and  $>0.0003$  mm. in diameter) is nearly identical with that of the entire colloid separate ( $<0.002$  mm. in diameter), one may assume that

TABLE 10

*Minerals in coarse colloid separate as revealed by specific gravity and X-ray analyses, and amounts of these minerals and potash in the entire colloid separate of the Hagerstown silt loam, Pennsylvania station*

PLAT SAMPLED	COARSE COLLOID SEPARATE $<0.002$ AND $>0.0003$ MM IN DIAMETER				AMOUNTS IN ENTIRE COLLOID SEPARATE $<0.002$ MM. IN DIAMETER CALCULATED ON BASIS CONTENT OF COARSE COLLOID SEPARATE		
	Specific gravity of mineral fractions	Percentage total potash in the mineral fractions	X-ray analysis		Pounds per acre of mineral fractions	Pounds per acre of potash in mineral fractions	Pounds per acre excess potash in KCl plat over that in check plat in the mineral fractions
			Minerals shown by X-ray analysis	Remarks			
Check plat received no KCl	$<2.667$	1.74	Quartz	No muscovite lines visible	428,086	7,449	..
	$>2.667$	3.90	Muscovite	Muscovite lines extended only through first order	33,711	1,315	..
KCl plat received equivalent of 3158 pounds per acre of potash	$<2.667$	2.05	Quartz	No muscovite lines visible	398,671	8,173	724
	$>2.667$	4.67	Muscovite	Muscovite lines extended through second order	73,451	3,430	2,115

their mineral fractions, as regards potash, are probably similar. Calculated on this basis, the heavy mineral fraction of the entire colloid separate of the potassium chloride plat contains 2,115 pounds of potash in excess of that contained in a similar fraction of the check plat. This is a quantity that almost exactly accounts for the 2,222 pounds of potash added as a fertilizer that could not be accounted for in the sum of the potassium removed by plants and that remaining in the soil in available form. This 2,115 pounds of excess potash is much more than the entire amount found in the corresponding heavy mineral fraction of the check plat, and hence it could hardly be argued that this came largely from the approximately one-fifth more feldspar potash

that exists in the potassium chloride plat over that in the check plat, because that would lead to the implication that more potash came from this one-fifth portion of feldspar than from five times as much feldspar in the case of the check plat.

Thus the conclusion which seems inevitable is that when potassium is added to a soil containing the usual kind of colloidal matter, a portion of it is converted into muscovite, and this potassium is no longer readily available to plants.

#### *Theories regarding the formation of muscovite in the soil*

That muscovite is formed in clays, shales, and slates as a secondary mineral, was first suggested by Hutchings (10, 11, 12) in 1890. He states:

And it is quite reasonable to suppose that when kaolin is deposited together with much feldspar, biotite, muscovite, etc., the changes which afterwards take place, involving as they do a micaceous alteration of the feldspar with liberation of alkaline silicate in solution, may effect a conversion of more or less of the kaolin into mica, a process chemically quite simple, and quite probable under the conditions of pressure and temperature we know to have existed.

Since it has been noted that heavy lime additions to soils may cause a decrease in available potassium, probably through fixation, another explanation can be offered for the formation of the muscovite in soils. By the addition of lime, potassium carbonate is forced into solution, and reaches a considerable concentration as the soil dries. This alkali carbonate attacks colloidal silica, colloidal alumina, and colloidal silicates in general, with the ultimate result that muscovite is formed either directly or through the reaction of the intermediate compounds. It should, however, be noted that, since fixation takes place when KCl is added to acid soils, alkalinity does not appear to be a necessary condition for fixation. The influence of lime and sodium carbonate could be explained as being due to the fact that they tend to keep the potassium out of the exchange material and hence in active condition to react with some form of colloidal silicate so as to form muscovite.

Other theories could be proposed, but the findings to date do not seem to warrant further speculation.

#### *Practical applications of the results reported herein*

Very likely there have been instances where the application of potash fertilizers has not given full crop response because of the high fixing capacity of the soil for potash.

From the standpoint of applying potash fertilizers under field conditions so as to reduce to a minimum the amount of potash that would be lost by fixation in difficultly available form, three practices are suggested: (a) frequent moderate applications instead of less frequent large applications of potash fertilizer, (b) application of the fertilizer to a greater depth than usually practiced, so as to get below the surface zone, which is subjected to frequent wetting and

drying, and (c) localized application of the fertilizer in the hill or row as to reduce the opportunity for fixation.

It should be noted in this connection that occasional burning and drying of a soil increases the immediate supply of readily available plant nutrients, including potash, as a result of their liberation from organic and possibly certain inorganic constituents. The processes involved are quite different from those discussed in this paper; for a detailed discussion and extensive review of the literature regarding this phase of the subject the reader is referred to the papers of Steenkamp (25, 26, 27).

#### SUMMARY

The purpose of this investigation was to study the nature of potash fixation, and to determine, if possible, the identity of the difficultly available compound formed when soluble potassium salts are added to soils. Samples of soils were treated with varying quantities of potassium bicarbonate or potassium chloride, moistened with water, and either allowed to dry slowly at room temperature or were alternately wetted and dried a number of times at 70°C. In order to determine the identity of the difficultly soluble mineral formed, a mechanical, mineralogical, chemical, and X-ray study was made of a sample of Hagerstown silt loam which had received a total of 5,000 pounds per acre of potassium chloride during the past 50 years, about half of which could not be accounted for in the potassium removed by the plants and remaining in the soil in available form. The results of the investigation are as follows:

Alternate wetting and drying of soils treated with soluble potassium salts caused rapid fixation of the potassium in a non-replaceable form. When these soils were kept continuously moist, very little fixation of this kind took place.

Ten alternate wettings and dryings usually sufficed to cause a maximum amount of fixation.

In four soils investigated, the ultra clay fraction contained the lowest percentage of total potash of any of the fractions, but, on the other hand, was by far the most active in fixation.

One hundred soils, treated with 1,000 pounds of potash per acre, showed a capacity to fix potash from nothing to all that was added.

Fixation is dependent on the nature of the colloids as well as the quantity. A lateritic soil, containing around 90 per cent colloid, did not fix potash, whereas Miami silt loam, containing only 24 per cent of colloid, fixed 495 pounds per acre out of 1,000 pounds added.

Potash fixation was reduced by leaching soils with HCl, and increased by leaching them with Na<sub>2</sub>CO<sub>3</sub>.

Most soils, alternately wetted and dried after treatment with 3 tons of calcium hydroxide per acre, were found to fix more potassium than those not treated with calcium hydroxide.

Synthetic mixtures of alumina gel, silica gel, calcium hydroxide, and sand did not fix potash.

Mineralogical, chemical, and X-ray analyses of Hagerstown silt loam from the Pennsylvania station experimental plats, some of which had received 5,000 pounds per acre of potassium chloride over a period of 50 years, led to the conclusion that a portion of the added potassium had reacted with colloidal silicates and become fixed in the form of muscovite, thus converting added available potassium into difficultly available form.

From a practical standpoint it would seem to be advisable to make frequent moderate applications of potash fertilizer rather than less frequent large applications, and also to apply the potash to a greater depth than is usually practiced, in order to lessen the influence of alternate wetting and drying. Localized application would also tend to reduce fixation.



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## THE DISTINCTION BETWEEN MAGNESIUM ABSORBED AND THAT EXCHANGEABLE, FOUR YEARS AFTER LYSIMETER INCORPORATIONS OF OXIDES AND CARBONATES

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In certain cylinder experiments, begun in 1913, it was found that the fate of heavy incorporations of hydroxy magnesium carbonate differs from that of equivalent incorporations of calcium carbonate. The "light" magnesium carbonate was absorbed more rapidly and also more extensively (8). In contrast with the large increases in carbonates found 8 weeks after incorporation of burnt lime, hydrated lime, and calcium carbonate at the rate of 16,000 pounds of CaO per acre, only meager carbonate residues were found for the equivalent incorporations of hydroxy magnesium carbonate. Subsequent to the early absorption of the magnesium carbonate, with and without manure, a marked temporary toxicity developed (9). In later supplemental studies, with tobacco, it was determined that additions of caustic magnesia reacted extensively with silicic acid (6).

Since the heavy additions of magnesium carbonate used in the cylinder experiments had been quickly and completely absorbed by the slightly acid and well-buffered Cumberland loam, the study was continued and amplified to determine the ultimate absorption-capacity of that soil. For this purpose a 44-unit lysimeter installation was provided to compare the absorptions from seven calcic and magnesian materials at the CaO-equivalent rates of 8, 32, and 100 tons so that ultimately a balance could be struck between the amounts added, those leached, and those residual in the carbonate and non-carbonate forms. During the first year of this supplemental lysimeter study it was again found that the absorption of the magnesium carbonate was more rapid and more extensive than that of calcium carbonate.

When the lysimeter experiment was inaugurated, the crystalline normal carbonate could not be obtained, and it was known that the precipitated carbonates used in the cylinder and lysimeter studies were combinations of  $MgCO_3$  and  $Mg(OH)_2$ . It was assumed, however, that the  $CO_2$ -enriched atmosphere and the carbonated free water of the soil would gradually convert the  $Mg(OH)_2$  contents of the hydroxy carbonates into  $MgCO_3$  and that the unabsorbed fractions of the additions would ultimately be present as the normal carbonate. From attempts to determine their fate at intervals during a 12-year period, it appeared that the unabsorbed fractions of the heavier additions of the hydrated carbonate continued as such. This was true, in spite

of the fact that large quantities of magnesium bicarbonate had passed out in the leachings. It therefore seemed necessary to provide substantial quantities of the magnesia-treated soils for laboratory study. Samples of such proportions could not be removed from the lysimeters of the long-period experiment without involving correction-computations and vitiating disruptions, especially in the case of the subsoil tanks. The initial lysimeter installation was therefore supplemented by additional tanks, the entire contents of which were to be available for laboratory study after an appropriate period of exposure, and the soils of this supplemental set of lysimeters were used in the present study.

#### EXPERIMENTAL

*Objectives.* This 9-unit adjunct lysimeter project was inaugurated in July 1925, to run for a period of 4 years. The outgo data have been reported (7). The present contribution deals with the influence of form and rate of magnesian treatments upon (a) extent of absorption, (b) nature of the absorbed magnesia as reflected by its exchangeability and solubility in dilute acids, (c) the ions replaced by the absorbed magnesium, and (d) any corollary chemical effects.

*Soil and treatments.* The soil was the same Cumberland loam used in the previous cylinder and lysimeter experiments. The five magnesian materials, "light" and "heavy" oxides, "light" and "heavy" hydroxy carbonates, and the crystalline normal carbonate,  $\text{MgCO}_3 \cdot 3\text{H}_2\text{O}$  were used. The last-named material had been prepared in the laboratory and identified. It was used because of its high solubility, as compared with the natural carbonate, magnesite. It is not ordinarily listed as a chemical, but it was ultimately obtained in quantities sufficient for the heavy lysimeter treatments. The materials were thoroughly incorporated with the soil before placement in the lysimeters and no subsoil stratum was used. On the basis of CaO equivalence per 2,000,000 pounds of soil, the experimental set-up, with a rainwater blank, was as follows:

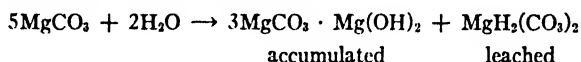
Unit 200	Untreated soil
Unit 201	"Light" magnesium oxide, 8 tons
202	"Heavy" magnesium oxide, 8 tons
Unit 203	"Light" precipitated magnesium carbonate, 8 tons
204	"Heavy" precipitated magnesium carbonate, 8 tons
205	Crystalline normal magnesium carbonate, 8 tons
Unit 206	"Light" magnesium oxide, 32 tons
207	"Light" precipitated magnesium carbonate, 32 tons
208	Crystalline normal magnesium carbonate, 32 tons

At the end of the 4-year period of leaching by rainfall only, the soils were removed from the lysimeters and thoroughly mixed, to provide substantial 0.5-mm. samples for the analyses given in tables 1-6. Sufficient opportunity was thus afforded for extensive reaction between the soil and the added magnesian materials and, presumably, for complete carbonation of the magnesium oxide.

*Presentation of data.* The results are given in terms of milliequivalents of magnesium. The carbonate residues from the five 8-ton oxide and carbonate additions were small and the magnesium increases were therefore practically the same as the respective amounts absorbed, or fixed. The crystalline carbonate unit 205 will therefore be taken to represent the treatments at the 8-ton rate.

To compute the absorptions from the several magnesian materials, it was necessary to determine the final occurrences of both magnesium and carbonate  $\text{CO}_2$ . In making a comparison between the increases in  $\text{CO}_2$  derived from the several additions it was also necessary to assume a common ultimate composition for the residues of magnesium carbonate. Opportunity to differentiate, with some degree of certainty, between the forms that make up the unabsorbed fractions of the added magnesian materials was therefore limited to the 32-ton units, all of which contained substantial carbonate residues.

The leaching data (7) indicated that the heavy outgo of magnesium bicarbonate from the normal carbonate brings about an accumulation of the hydroxy carbonate, as represented by the equation:



As previously noted, it also appeared that the incorporations of the hydrated carbonates, represented by the formula  $3\text{MgCO}_3 \cdot \text{Mg}(\text{OH})_2 \cdot 3\text{H}_2\text{O}$ , had not been converted into the normal carbonate,  $\text{MgCO}_3 \cdot 3\text{H}_2\text{O}$ . In accounting for the magnesium present in the carbonate form, it therefore seemed logical to assume that the hydrated carbonate would be the one more likely to be present. Under this assumption, the unabsorbed magnesium is  $33\frac{1}{3}$  per cent greater than the amount that would be computed from a simple  $\text{CO}_2$  equivalence. The  $\text{CO}_2$  results from the oxide additions were computed in the same manner, but the relationships in the oxide systems are more involved. It is well known that properties of magnesium oxide are specific for the different types obtained from different carbonates and that calcination temperatures materially affect the reactivities of the calcined product. A part of the unabsorbed fraction of added oxide may continue as such, a part may be present as the hydrated oxide, and the remainder as the carbonate. This point will be considered further in a discussion of the computations for the absorptions.

*Extent of magnesia absorptions.* The increases in magnesium, as determined by digestion with  $\text{HCl}$ , sp. gr. 1.115, and the carbonate occurrences, are given in table 1. The fundamental data of this table are used as the basis of comparison for all of the leachate and extraction data presented in tables 2-6. As previously explained, table 1 shows the quantities of "unabsorbed" magnesium present in the postulated form of  $3\text{MgCO}_3 \cdot \text{Mg}(\text{OH})_2 \cdot 3\text{H}_2\text{O}$ . The values for "absorbed" magnesia are shown in the last column, and are the respective differences between the values for "increase" and "unabsorbed."

It is evident that the absorption from each of the 32-ton treatments greatly

exceeds that found for the 8-ton rate. The maximal absorption capacity of the heavy clay loam for magnesium for the 4-year period of moist contact and dynamic condition was about 2 per cent  $\text{CaCO}_3$  equivalence, or 20 tons per 2,000,000 pounds of soil. The computation of 3.102 per cent, or 62.04 m.e., for absorption in the oxide unit 206 is obviously in excess of the true value, because from the determined carbonate occurrence of 0.599 per cent it is evident that a considerable part of the added oxide had not been converted to the carbonate. It has been shown (7) that in spite of this incomplete transition of oxide to carbonate, the  $\text{CaCO}_3$ -equivalence of the carbonates leached from the 32-ton addition of magnesian oxide by the rainwater during 4 years far exceeded that leached from the equivalent treatment of hydrated carbonate—12.93 tons against 10.59 tons. Hence, in spite of the fact that the oxide residue from unit 206 was bathed copiously with solutions of magnesium bicarbonate for 4 years, the gain in carbonates was only 30 per cent of the carbonate residue from the equivalent addition of hydroxy carbonate. The carbonate gain for the oxide was only 0.599 per cent against 3.90 per cent for magnesium “increase,” whereas the residual carbonate from the hydroxy carbonate of unit 207 was 1.942 per cent along with the total magnesium gain of 4.29 per cent. The ratio between carbonate increase and total magnesium increase was therefore 1:6.5 for the oxide addition, as compared with corresponding ratios of 1:2.16 and 1:3.17 for the equivalent treatments of the hydroxy carbonate and the normal carbonate, respectively.

Although the heavy addition of oxide yielded large quantities of magnesium bicarbonate to the rain water leachings over the 4-year period, as shown previously (7), the air-dried uncarbonated residue was not active toward a moist absolute  $\text{CO}_2$  atmosphere. No increase in carbonates was found for the soil of either unit when 15-gm. air-dried charges of the oxide unit 206 and the hydrated carbonate unit 207 were bathed for 72 hours with currents of  $\text{CO}_2$  that previously had passed slowly through a column of water in “worm” scrubbers. These two soils were then mixed with pure quartz, and the mixtures were moistened and accorded the same contact with an atmosphere of  $\text{CO}_2$  for a 72-hour period. The moist mixture of the oxide unit 206 gained 0.062 per cent  $\text{CO}_2$ , on the basis of original air-dry soil. That is, after this subsequent carbonation, the carbonate-accumulation value that had been found for the 32-ton oxide unit at the end of 4 years of lysimeter exposure was increased from 0.599 per cent to 0.740 per cent. Hence, on the assumption that the amount of magnesium absorbed from the oxide was the same as that absorbed from the hydroxy carbonate—a liberal assumption—the unabsorbed residue of the oxide addition was 36 per cent uncarbonated. There was no difference, however, between the carbonate content of unit 207 before and after the additional exposure to a moist atmosphere of  $\text{CO}_2$ . It is therefore evident that the uncarbonated oxide residues continued to show a slow transformation to carbonates, and that the hydrated carbonate was not transformed into the normal carbonate.

TABLE 1  
*Magnesium residues in a Cumberland clay loam 4 years after incorporations of equivalent additions of oxide and hydroxy and normal carbonates of magnesium in outdoor lysimeters*

UNIT	MATERIALS INCORPORATED			MAGNESIUM AS PER CENT $\text{CaCO}_3$ AND AS MILLIEQUIVALENTS											
	Form	Amount*		Soluble in 1 N HCl						Carbonates		Unabsorbed†		Absorbed	
		Per cent $\text{CaCO}_3$	m. e.	Found		Increase									
				per cent	m. e.	per cent	m. e.	per cent	m. e.	per cent	m. e.	per cent	m. e.		
200	None			0.65	13.00										
205	Crystalline normal carbonate‡	1 4285	28.57	1.25	25.00	0.60	12.00	0.075	1.50	0.100	2.00	0.500	10.00		
206	"Light" oxide	5 7140	114.28	4.55	91.00	3.90	78.00	0.599	11.98§	0.798	15.96§	3.102	62.04§		
207	"Light" precipitated carbonate	5 7140	114.28	4.94	98.80	4.29	85.80	1.942	38.84	2.589	51.78	1.701	34.02		
208	Crystalline normal carbonate	5 7140	114.28	4.09	81.80	3.44	68.80	1.086	21.72	1.448	28.96	1.992	39.84		

\* Equivalent to rates of 8 and 32 tons CaO per 2,000,000 pounds of soil.

† Computed from  $\text{CO}_2$  data, on the assumption that the unabsorbed fraction of the added magnesium is present as  $3 \text{MgCO}_3 \cdot \text{Mg}(\text{OH})_2 \cdot 3\text{H}_2\text{O}$ .

‡ Representative of four other practically completely absorbed additions of "light" and "heavy" oxides and their corresponding carbonates.

§ The value computed for "absorbed" is obviously high and that computed for "unabsorbed" is low because of the incomplete carbonation of the added oxide.

TABLE 2  
*Magnesium recoveries by normal ammonium chloride leachings of the residues from equivalent additions of oxide and hydroxy and normal carbonates, 4 years after incorporations in outdoor lysimeters*

UNIT	MATERIALS INCORPORATED		MAGNESIUM EXTRACTED FROM 25-GM CHARGES BY SUCCESSIVE 500-ML LEACHATES											
			Form	Amount*		1	2	3	4	5	6	7	8	Total
	Per cent CaCO <sub>3</sub>	m e												
				m e	m e									
200		None			1 12	0 62	0 28	0 30	0 14	0 02	0 10	0 06	2 64	..
205		Crystalline normal carbonate†	1 4285	28 57	9 28	1 40	0 82	0 66	0 66	0 14	0 14	0 10	13 20	88 0
206		"Light" oxide	5 7140	114 28	31 36	9 22	5 98	5 56	3 56	2 18	1 88	1 38	61 12	75 0
207		"Light" precipitated carbonate	5 7140	114 28	57 50	6 12	4 06	3 56	2 24	1 38	1 16	0 82	76 84	86 5
208		Crystalline normal carbonate	5 7140	114 28	38 18	6 42	4 52	4 02	2 50	1 46	1 36	0 78	59 24	82 3

\* Equivalent to rates of 8 and 32 tons CaO per 2,000,000 pounds of soil.

† Representative of four other practically completely absorbed additions of "light" and "heavy" oxides and the corresponding carbonates.



## MAGNESIUM RECOVERIES

*Ammonium chloride leachings.* The results of table 2 show the recoveries from each of the five units of table 1, by eight successive 500-ml. leachings with a neutralized normal solution of ammonium chloride, according to the Hissink procedure (2). Five leachings were required to reduce the yields from the 8-ton unit 205 to the level found for the control, and to effect a recovery comparable to that shown by the strong acid digestion of the same unit in table 1. In the first leachates, wherein direct solvent action was at play, the recoveries from the two 32-ton carbonate additions were considerably greater than the recovery from the oxide, although the oxide residue exceeded that of the crystalline carbonate. The ultimate recoveries assumed the same order as that of the

TABLE 3

*Magnesium recoveries from additions of oxide and hydroxy and normal carbonates made to an air-dry Cumberland clay loam immediately preceding leaching with normal ammonium chloride*

MAGNESIUM ADDITIONS TO ACID SOIL CONTROL*			MAGNESIUM EXTRACTED FROM 25-GM CHARGES BY SUCCESSIVE 500-ML. LEACHATES								
Form	Amount		1	2	3	4	5	6	7	8	Total
	Per cent CaCO <sub>3</sub>	m e	m e	m e	m e	m e	m e	m e	m e	m e	m e
"Light" oxide.....	1 398	27 96	27.34	0.76	0 42	0.22	0.16	0 06	0 08	0 08	29 12
Light precipitated carbonate .	1 448	28 96	29 42	0 38	0 24	0 26	0 08	0 06	0 26	0 08	30.78
Normal crystalline carbonate.	1 658	33.16	34 08	0 48	0 32	0 18	lost	0 04	0.10	0 46	35 66

\* Same as unit 200, tables 1, 2, and 6.

total residues. Appreciable quantities of magnesium were recovered from each of the heavy treatments, even in the eighth leachate. Apparently the continued leachate recovery was due to the fact that a large part of the absorbed magnesium was resistant to exchange for the NH<sub>4</sub> ions.

To demonstrate that this extended recovery was not attributable to protracted direct solvent action of the neutralized ammonium chloride solution upon the different unabsorbed residues of the several magnesian additions, the following procedure was carried out. The light oxide, the light hydroxy carbonate, and the normal carbonate were added separately to the air-dried original soil in amounts comparable with the respective quantities of the same three materials that remained after 4 years of exposure to leaching. The several fortified charges of the air-dry control soil were then immediately extracted with a normal solution of ammonium chloride, exactly as in the procedure that had been followed in obtaining the results of table 2. The data of table 3 demonstrate that the laboratory additions were entirely removed

by the first 500-ml. leachates. The subsequent leachates from the fortified charges were at the low levels shown for the untreated soil in table 2. When the totals given in the last column of table 3 are corrected for the magnesium leached from the untreated soil, table 2, the recoveries agree with the laboratory additions made to the air-dried control. When these results are used as criteria for the identical ammonium chloride leachings from the 32-ton lysimeter units, it is concluded that the extended magnesia recoveries of table 2—evidenced even in the eighth 500-ml. leachate—were due to the continued removal of absorbed magnesia from the absorption complex, rather than to meager and extended dissolving of the unabsorbed magnesium residues. It is certain that the exchange between the tenaciously held magnesium and the  $\text{NH}_4$  ions of the ammonium chloride solution was not so readily effected as that found for *native* supplies of exchangeable magnesium with limited leachates of 300 to 500 ml., as in the Hissink method (2).

The exchangeability of the absorbed magnesia was determined by the method proposed by Kelley and Brown (3). The amounts of ammonia exchanged and retained by the five soils of tables 1 and 2 during 4-liter leachings with a normal solution of ammonium chloride were:

	CONTROL, NO ADDITION	UNIT 205, 8 TONS NORMAL CARBONATE	UNIT 206, 32 TONS LIGHT OXIDE	UNIT 207, 32 TONS LIGHT PPT CARBONATE	UNIT 208, 32 TONS NORMAL CARBONATE
m.e. ....	8 8	9 0	9.0	9 2	9 0

The exchange capacity of the untreated soil was practically the same as that found for the four magnesia-treated lysimeter soils. These uniform ammonium recoveries are only about 55 per cent of the equivalence of the magnesium + calcium removed from the 8-ton unit 205 by the leaching procedure. In a comparison of the varying and extended recoveries of magnesium effected by the ammonium chloride leachings and the exchange capacity results by the Kelley and Brown procedure after the 4-year period of contact, it is evident that the exchange capacities of the several units are not correlated with the magnesium absorptions. Only a small fraction of the large quantities of the absorbed magnesium was in the exchangeable state, in the accepted meaning of the term. Hence, any upbuilding, or enrichment, of magnesium in the exchange complex that may have transpired as a result of the magnesian additions was not registered by either the Hissink procedure or that of Kelley and Brown.

The difference between the magnesium absorption and the ammonium exchanged by the magnesium-enriched soils during the leachings with neutralized solution of ammonium chloride may be attributed, of course, to the influence of pH values. The absorption of  $\text{NH}_4$  ions from the solution of ammonium chloride represents the absorption capacity of the soil for a solution having a pH of 7.0, whereas the magnesium absorption in the lysimeter units reflects

the absorption capacity for magnesium at a pH of approximately 9. Another important difference is that of the time of contact.

*Ammonium chloride extractions.* Since complete recoveries of the absorbed magnesium were not obtained by the leachings with normal solutions of ammonium chloride, the 32-ton units 206, 207, and 208 were subjected to repeated extractions with both ammonium chloride and dilute acids, and also to successive leachings with dilute acids, according to the Gedroiz method (1).

The recoveries obtained by successive agitated overnight extractions with a neutralized normal solution of ammonium chloride are given in table 4. The

TABLE 4

*Magnesium recoveries by normal ammonium chloride extractions of the residues from equivalent additions of oxide and hydroxy and normal carbonates 4 years after incorporations in outdoor lysimeters*

UNIT	MATERIALS INCORPORATED		MAGNESIA RECOVERIES BY SUCCESSIVE 500-ML. OVERNIGHT EXTRACTIONS OF 25-GM. CHARGES						
	Form	Amount*		1	2	3	4	Total	Recov- ery†
		Per cent CaCO <sub>3</sub> eq.	m. e.						
200	None	...	...	m. e. 0 54	m. e. 0 04	m. e. 0 04	m. e. 0 04	m. e. 0 66	per cent .
205	Crystalline normal carbonate‡	1 4285	28 57	8 04	0 74	0 40	0 48	9 66	81
206	"Light" oxide	5 7640	114 28	30 64	10 64	6 28	5 30	52 86	68
207	"Light" precipitated carbonate	5 7140	114 28	52 08	10 88	5 04	4 04	72. 04	84
208	Crystalline normal carbonate	5 7140	114 28	36 60	10 24	5 50	4 04	56 38	82

\* Equivalent to rates of 8 and 32 tons CaO per 2,000,000 pounds of soil.

† Based on the "increase" values shown in table 1.

‡ Representative of four other almost completely absorbed additions of "light" and "heavy" oxides and the corresponding carbonates.

magnesium removed from the untreated soil by these four extractions was less than the amount removed by the first four of the eight leachates of table 2. The same holds true for the soil of the 8-ton unit 205. The same comparison shows a close agreement for the oxide and hydroxy carbonate units 206 and 207, with a difference of 3.24 m.e. in the case of the normal carbonate unit 208.

The total recoveries obtained by the eight leachates were in excess of those obtained by the four extractions in all cases, although the results for the carbonate units 207 and 208 did not vary widely. It is evident that the smaller number of extended agitated extractions were almost as effective as twice the number of leachings, but full recoveries of total residues were not obtained by either of the procedures.

*Acid digestion-leachings.* In the procedure for acid leaching, following a

digestion period, 25-gm. portions of soil were treated in 250-cc. beakers with an amount of 0.1 *N* HCl just sufficient to decompose the determined amounts of carbonates present. The beakers containing soil and 0.1 *N* acid were placed on a water bath at a temperature of 70°C. for 1 hour and permitted to stand overnight. The acid-digested soils were then filtered and leached as prescribed by Gedroiz, except that 0.02 *N* HCl was used in order to minimize the solubility of manganese and of aluminum. Four successive 500-ml. volumes of leachates were collected, and each leachate was analyzed for both magnesium and calcium. The data of table 5 show that the magnesium recoveries by this acid digestion-leaching treatment were not equal to those obtained by the strong acid digestions of table 1. Nevertheless, the leachings with 2 liters of 0.02 *N* acid gave recoveries materially greater than those obtained by ammonium chloride in the 4-liter leachates shown in the last column of table 2, and by the four successive extractions of table 4.

TABLE 5

*Magnesium recoveries by 0.02 N HCl leachings of the residues from equivalent additions\* of oxide and hydroxy and normal carbonates 4 years after incorporations in outdoor lysimeters*

UNIT	MATERIALS INCORPORATED	MAGNESIUM EXTRACTED FROM 25-GM. CHARGES BY SUCCESSIVE 500 ML. LEACHATES†					
		1	2	3	4	Total	Recovery†
		m.e.	m.e.	m.e.	m.e.	m.e.	per cent
206	"Light" oxide	30 52	15 32	14 46	11 28	71 58	92
207	"Light" precipitated carbonate	57 98	12 52	8 84	2 40	81 74	95
208	Crystalline normal carbonate	40 60	9 62	9 56	3 58	63.36	92

\* Equivalent to 5.714 per cent  $\text{CaCO}_3$ , 114.28 m.e. and rate of 32 tons  $\text{CaO}$  per 2,000,000 pounds of soil

† Based on the "increase" values shown in table 1.

*Agitated acid extractions.* The repeated acid extractions with continuous agitation were carried out as follows: Ten-gram charges of the five units of tables 1, 2, and 4 were subjected to four successive 16-hour, or overnight, agitated extractions in the cold with 0.02 *N*  $\text{HNO}_3$ . In each of the initial extractions, sufficient 0.05 *N* acid was used to decompose the carbonates and still give a residual strength of 0.02 normality. The agitated suspensions were then thrown on Büchner funnels and washed three times with distilled water, and the soil was returned to the original flask with a measured quantity of 0.02 *N*  $\text{HNO}_3$  for each succeeding extraction. The four successive filtrates were made to volume, and aliquots were used for the determinations of the "acid-neutralized" and "magnesia-extracted" results of table 6.

The "recovery" values of table 6 give the respective variations from the "increase" values shown for the strong HCl extractions of table 1. In each instance the full magnesium "increase" obtained by the strong acid was also obtained as "total" by the four extractions with 0.02 *N* acid and even by the

TABLE 6  
*Acid neutralized by, and magnesium recoveries from, absorbed and unabsorbed residues during four agitated overnight extractions, 4 years after equivalent incorporations of oxide and hydroxy and normal carbonates in outdoor lysimeters*

UNIT	MATERIALS INCORPORATED		RESULTS OF SUCCESSIVE 500 ML EXTRACTIONS OF 10- <small>CM.</small> CHARGES WITH 0.02 NORMAL HNO <sub>3</sub>											
			Acid neutralized				Magnesium extracted				Magnesium recovery			
	Form	Amount*	1	2	3	4	Total	1	2	3	4	Total	m. e.	per cent
			m. e.	m. e.	m. e.	m. e.	m. e.	m. e.	m. e.	m. e.	m. e.	m. e.	m. e.	m. e.
200	None	.....	5 80	0 40	1 00	1 00	8 20	1 08	0 42	0 24	0 32	2 06	.....	.....
205	Crystalline magnesium carbonate†	28 57	18 60	2 80	0 00	0 20	21 60	12 04	0 94	0 44	0 26	13 68	+1.6	+ .08
206	"Light" oxide	114 28	82 20	3 00	1 20	0 00	86 40	76 84	1 74	0 64	0 36	79 58	+1.6	+ .08
207	"Light" precipitated carbonate	114.28	87 80	4 20	1 60	1 40	95 00	83 62	2 40	0 82	0 36	87 20	+1.4	+ .07
208	Crystalline normal carbonate	114.28	73 40	2 40	1 00	0 00	76 80	68 90	1 76	0 72	0 28	71 66	+2 8	+ .14

\* Equivalent to rates of 8 and 32 tons CaO per 2,000,000 pounds of soil.

† Representative of four other practically completely absorbed additions of "light" and "heavy" oxides and the corresponding carbonates.

first two extractions with the dilute acid. Allowing for the cumulative analytical error, attributable to incomplete precipitation of calcium, in the eight analysis of solutions rich in magnesium and poor in calcium, the results by the two methods may be considered as being in close agreement. Hence, in these experimental soils, there was no appreciable difference between the solubility found for the absorbed magnesium by strong acid digestion and the aggregate dilute acid-extraction value that is ordinarily designated as "exchangeable," and as determined by the Gedroiz method.

*"Acid neutralized."* The determinations of the quantities of acid neutralized by the magnesium residues in the several soils of table 6 served to record the progress of the extractions before the extracts could be analyzed and they also served as checks upon the respective sums of the several cations. Each "acid neutralized" value exceeds the corresponding value for magnesium extracted but is within 0.1 per cent of the directly determined corresponding sum of the extracted magnesium and (unreported) calcium.

It has been pointed out that the amount of calcium extracted from each lysimeter unit was greater than that extracted from the untreated soil. This apparent discrepancy is accounted for readily. The calcium content of each treated unit was actually in excess of that of the untreated control soils, and for two reasons. The added magnesian materials exerted a marked repressive effect upon the outgo of calcium during the 4-year period of lysimeter exposure. In terms of  $\text{CaCO}_3$ , the averaged outgo of calcium from the three 32-ton treatments was 1,373 pounds less than the calcium outgo from the untreated soil (7). Furthermore, some calcium was introduced, even by the "c.p." magnesian materials. The calcium impurities in the light oxide, carbonate, and normal carbonate were 3.64, 1.26, and 0.22 per cent, respectively, by the methyl-ethyl alcohol method which was used in making all of the magnesia determinations reported. It should be noted that when small quantities of calcium occur with preponderant quantities of magnesium, calcium cannot be determined by the conventional oxalate method of precipitation (5).

*Other changes in solubility.* It seemed desirable to determine the effects exerted by the magnesian materials upon the solubility of the soil complexes. The acid extracts of table 6 were therefore analyzed for iron and aluminum. The amounts of aluminum extracted from the soil that received the heavy magnesian treatments were much greater than those extracted from the untreated soil. Increases in extractable aluminum were noted especially in the first two extractions, diminishing in the third extraction, and they were not registered in the fourth extraction. The sesquioxide totals removed by the first three extractions were 0.366 per cent for the untreated soil, and 0.405, 0.557, 0.492, and 0.545 per cent for units 205, 206, 207, and 208, respectively. The proportion of iron to aluminum in the extracts from the untreated soil was decidedly different from that found in the extracts from the magnesia-treated units. The ammoniacal precipitates of the acid extractions from the magnesia-treated units were composed almost entirely of alumina, whereas

those from the untreated soil contained a high proportion of iron. Although it has been shown that additions of caustic magnesia will react with colloidal silica that may be present in the soil (6), the foregoing observations indicate



FIG. 1. COMPARISON OF FOUR PROCEDURES IN EFFECTING RECOVERIES OF THE MAGNESIUM RESIDUES FROM 32-TON  $\text{CaO}$  ADDITIONS OF OXIDE, HYDROXY CARBONATE, AND NORMAL CARBONATE 4 YEARS AFTER INCORPORATIONS IN OUTDOOR LYSIMETERS

Full "increase" of table 1 used as 100 per cent.

1. Eight 500-ml. *leachings* with  $N \text{ NH}_4\text{Cl}$ , table 2.
2. Four 500 ml. *extractions* with  $N \text{ NH}_4\text{Cl}$ , table 4.
3. Four 500-ml. *leachings* with  $0.02 N \text{ HCl}$ , table 5.
4. Four 500-ml. *extractions* with  $0.02 N \text{ HNO}_3$ , table 6.

that the absorbed magnesium had entered into the structure of the aluminum silicate complexes of the soil.

*Exchange capacity vs. magnesia absorptions.* The total exchange capacity of the Cumberland loam, as determined by the fixation of  $\text{NH}_4$  from a neutralized normal solution of ammonium chloride, was 8.8 m.e. This total value

was accounted for by 3.6 m.e. of exchangeable calcium, 1.12 m.e. of exchangeable magnesium, 0.48 m.e. of exchangeable potassium, negligible sodium, and 3.6 m.e. of exchangeable hydrogen, or 41 per cent unsaturation. Since the exchangeable calcium in each of the magnesia-treated lysimeter soils was more than that of the control after 4 years of leaching, because of the marked *decrease in calcium leached* from all of the magnesian treatments (7), and since all of the magnesian treatments also decreased materially the outgo of potassium (7), it is evident that only hydrogen replacement remains to explain a direct chemical absorption of magnesium. But the average absorption of magnesium shown for the two carbonate units 207 and 208 in table 1 was 1.847 per cent (36.94 m.e.) or more than 10 times the amount of exchangeable hydrogen registered by the ammonium chloride methods. All of this absorbed magnesium was recovered by agitated extractions with the very dilute acid, although not registered as the exchangeable form by widely used exchange methods. The usual exchange methods therefore fall far short of registering the amount of hydrogen replaced by the heavy additions of alkaline magnesium compounds in the Cumberland loam that may be taken as representative of the heavier types of well-leached residual soils of humid regions. Full recognition should be given to the importance of *time* as a factor in the extensive fixations.

If the replaceable-hydrogen determinations be taken as a measure of the readily exchangeable hydrogen, and the magnesium absorptions be taken as an actual measure of total replaceable hydrogen, it is evident that the Cumberland loam contains hydrogen ions that are held with different degrees of tenacity.

The incomplete recovery of the absorbed magnesium by the exchange reaction should not be taken to indicate that the solubility of the quantities absorbed from economic additions is so low as to prevent absorbed magnesium from entering readily into the free soil-water. On the contrary, the outgo of magnesium from such additions shows that the absorption complex is readily hydrolyzed—and more so than corresponding quantities of absorbed calcium.

The fact that the initial rapid disintegration of excessive additions of alkaline earth carbonates is followed by a subsequent slower decomposition during a long period of time had been determined at the Tennessee station (4) by another line of attack. For want of a better terminology, the more intensive initial reaction between soil and excesses of added carbonates was at that time designated as "Immediate Lime Requirement," whereas the extended reaction was designated as "Continuous Lime Requirement." The present experiments confirm the conclusion that there are present in the soil certain acid complexes that undergo rapid reaction with alkaline earths added in moderate or economic amounts, whereas there are other acid compounds that react only when excesses of carbonates are added and extended periods of contact are permitted. The two conditions, excessive additions and extended period of contact, were imposed in both the initial and present experiments.

It should be emphasized, however, that the speed of magnesium absorptions



from heavy additions of the more soluble alkaline magnesian carbonates was much greater than that found for chemically equivalent amounts of the less soluble carbonate of calcium. The mass-action effect of the more soluble magnesian materials apparently causes the heavy additions of magnesium to enter into the absorbed state to a much greater extent than that found for the less soluble calcium carbonate. The corollary fixation of calcium is under consideration for separate presentation.

A comparison of the recoveries registered by the several methods in the data of tables 2, 4, 5, and 6 is shown graphically in figure 1, the "increase" values of table 1 being taken as 100 per cent.

#### SUMMARY AND CONCLUSIONS

As a sequence to certain cylinder and lysimeter experiments relative to the specific absorptions found for seven calcic and magnesian materials, an adjunct lysimeter installation was used to determine the influence of form and amount of the "light" and "heavy" oxides and carbonates and the normal carbonate of magnesium upon the extent and nature of magnesium absorptions during a 4-year period.

The quantities of magnesium absorbed from 32-ton additions were much greater than those found for 8-ton treatments, but the minimal absorption greatly exceeded the exchange capacity registered by conventional methods.

The absorbed magnesium was found to be resistant to eight successive leachings, and also to four successive extractions, with a normal solution of ammonium chloride, the values obtained by the two procedures being comparable.

Since immediate additions equal to the unabsorbed residues were completely recovered by the first of eight ammonium chloride leachates, the prolonged recovery of the absorbed magnesium by multiple leachings was found to be attributable to a continued yield of magnesium from the absorption complex, and not to direct solvent action upon the unabsorbed magnesium residues.

The exchange capacities of the untreated soil and those of the magnesium-treated soils were found to be identical by the Kelly-Brown procedure.

Dilute acid extractions were found to be more efficacious than either leachings or extractions with ammonium chloride in effecting recoveries of absorbed magnesium.

Although none of the exchange methods registered quantities comparable to the amounts of magnesium actually absorbed, four overnight  $\text{HNO}_3$  extractions of 0.02 maintained normality, with concomitant "acid neutralized" determinations, showed respective recoveries equal to those obtained by digestion with strong  $\text{HCl}$ .

A definite increase in the alumina dissolved from the magnesia-treated units indicated that the added magnesium had disrupted the aluminum complex.

Since both the lysimeter history and the laboratory determinations for each

soil unit showed that neither calcium nor potassium had been replaced by the added magnesium, the magnesium absorptions are accounted for only by hydrogen replacement; but the exchangeable hydrogen by the conventional methods was only one-tenth the equivalent of the maximal magnesium absorption. Hence, in relation to and confirming results from a different method of attack, it was concluded that in the soil system are two types of acid complexes. One of these reacts readily with economic quantities of magnesian materials to form exchangeable complexes, whereas the other type will react much more extensively with excessive incorporations over an extended period, to form absorption complexes not measurable by conventional methods, although measured by successive agitated extractions with 0.02 *N* nitric acid.

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# CALCIUM CYANAMID AS A NITROGENOUS FERTILIZER<sup>1</sup>

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Calcium cyanamid, the first of the synthetic-nitrogen fertilizers, was introduced commercially in the early part of the twentieth century. That produced in America, and used in the experimental work reported in this paper, is grayish-black in color and contains 22 per cent nitrogen and calcium equivalent to 70 per cent hydrated lime. It is available in both pulverized and granular forms. The former is oiled to prevent undue dustiness. The latter is free of dust and consists of small pellets, passing a 12-mesh screen.

Considerable research has been conducted to determine whether calcium cyanamid will meet all the requirements of a nitrogenous fertilizer. The early literature dealing with its use is very inconsistent, excellent results having been obtained from its use in many instances, but unsatisfactory results or actual injury to the crop in others. More recent investigations show that when properly used, it gives satisfactory results.

When a new material having radically different characteristics is compared with a product the method of use of which has been standardized, the new material is often at a disadvantage. The present study was undertaken for the purpose of clarifying the situation with reference to calcium cyanamid. Care was taken in all of the experiments dealing with nitrogen availability to use the calcium cyanamid under conditions most favorable to its full efficiency.

The work here reported on calcium cyanamid was planned to give definite information on the following points:

- The availability of its nitrogen (crop yields in comparison with other nitrogenous fertilizers).
- The loss of nitrogen by leaching in comparison with other nitrogenous materials.
- Its effect on the physical and chemical properties of the soil.
- Its effect on the soil reaction.
- Its use as a weed controlling agent.

**Fertilizers used.** The calcium cyanamid used in these investigations was the regular commercial calcium cyanamid produced by the American Cyanamid

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Company at Niagara Falls. Fresh samples were obtained at frequent intervals. This material has approximately the following composition: calcium cyanamid 63 per cent, free hydrated lime 17 per cent, graphitic carbon 12 per cent, limestone impurities 4 per cent, and oil 4 per cent. The oil is replaced by calcium nitrate in the granular calcium cyanamid.

In all of the regular nitrogen-availability studies, a generous supply of superphosphate and muriate of potash was added to the soil at the time the nitrogenous fertilizers were applied, thus eliminating the possibility of a deficiency of phosphoric acid or potash.

*Methods of applying the fertilizers.* When nitrogenous fertilizers were used as a top-dressing, they were applied as evenly as possible by hand, early in the spring, and while the vegetation was dry.

All other applications were made about 1 week before the seeds were planted. Each fertilizer was thoroughly mixed throughout the soil in the pot, or to a depth of about 4 inches in cylinder and plot experiments.

*Preparation of samples and methods of analysis.* Crops were usually harvested at or near maturity. Samples were then dried in the hot air or electric oven, and they were ground for analysis.

The total nitrogen was determined by the official Kjeldahl-Gunning-Arnold method. Duplicate determinations were run on each sample, and if they did not check within 0.1 per cent nitrogen, the analysis was repeated in duplicate. One-gram samples were used for analysis.

#### PRESENTATION OF RESULTS

##### *Effect of continued use of various nitrogenous fertilizers on the crop yields*

A Sassafras-loam soil with a pH of 5.8 was screened and well mixed. Sixteen pounds of this soil was then placed into each of five 2-gallon coffee-urn-shaped glazed pots.

Each pot was treated with 1 gm. of triple-superphosphate (41 per cent) and 1 gm. of muriate of potash for each crop grown. The nitrogenous treatments were made at the rate of 429 mgm. of nitrogen per pot for each crop. Eleven crops were grown in each pot. At the end of the experiment, each pot had received the equivalent of about 1,000 pounds of nitrogen per 2,000,000 pounds of soil.

Table 1 shows the crop yields and the amount of nitrogen contained in each. This table also shows the total crop yield, and the milligrams of nitrogen contained in each of the 11 crops. The experimental data indicate that the several carriers of nitrogen differ considerably in their effect on crop yields if the applications of each material are repeated a number of times on the same soil. The highest total yield was obtained by the use of animal tankage, followed closely by calcium cyanamid. The nitrogen recovery figures place calcium cyanamid first with 65.9 per cent recovery, sodium nitrate second, tankage third, and ammonium sulfate last with a nitrogen recovery of 36.4 per cent.

Sturkie and Bailey (23) reported similar effects with the continued use of calcium cyanamid, sodium nitrate, and ammonium sulphate upon crop yields. Their plots were treated annually with 22.5 pounds of nitrogen per acre over a period of 20 years or a total of 450 pounds of nitrogen was applied. Five different crops were grown on each plot during the last three years of the test. When the total of these five crop yields, for each plot, is taken, and the yield produced by calcium cyanamid taken as 100, then the sodium nitrate rating is 73, no nitrogen 49, and ammonium sulphate 25.

TABLE 1

*Effect of various nitrogen\* fertilizers on nitrogen recoveries of 11 crops grown in succession on same soil*

SOIL TREATMENT	YIELDS IN DRY WEIGHTS												
	Corn	Corn	Corn	Corn	Corn	Wheat	Barley	Barley	Corn	Corn	Corn	Total 11 crops	
	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	
No nitrogen	11 3	9 8	5 5	6 6	9 5	1 7	2 0	1 4	10 7	6 5	14 7	79 7	
Sodium nitrate	25 0	18 0	18 0	12 0	14 4	5 2	4 4	1 2	9 0	10 5	24 0	141 8	
Ammonium sulfate	34 0	18 0	14 0	11 6	23 2	4 6	0 9	0 2	3 0	3 2	3 0	115 5	
Calcium cyanamid	26 5	17 2	19 0	13 4	19 0	6 7	4 7	1 8	14 3	15 0	30 0	167 6	
Animal tankage	25 0	17 1	16 5	15 5	19 1	5 0	4 5	1 7	17 0	15 0	33 1	169 5	

*Nitrogen contained in harvested crops*

	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	per cent recovery
No nitrogen	217	102	80	104	123	43	48	52	175	97	128	1,169	
Sodium nitrate	535	398	473	368	403	274	201	59	355	378	319	3,763	55 0
Ammonium sulfate	544	364	380	376	549	213	18	9	155	136	142	2,886	36 4
Calcium cyanamid	504	344	486	413	503	294	217	93	549	467	408	4,278	65 9
Animal tankage	355	246	326	387	389	188	196	74	595	477	417	3,650	52 6

\* Enough of each material to supply 1,000 pounds of elemental nitrogen per 2,000,000 pounds of soil was evenly divided among the 11 crops grown.

*Comparison of calcium cyanamid and sodium nitrate on wheat on small plots on various soil types*

The purpose of this experiment with wheat, conducted in square-yard plots (pl. 1, fig. 1) on five different soil types, was to compare yields and nitrogen recovery from the use of calcium cyanamid and sodium nitrate. Calcium carbonate was added to each soil to bring the pH between 6 and 7.

The data in table 2 show that the calcium cyanamid gave higher average crop yields than did an equivalent amount of sodium nitrate. It is quite probable that more of the fall application of nitrogen was retained by the soil in the case of the plots receiving the calcium cyanamid than in the case of those treated with nitrate.

TABLE 2

*Comparison of calcium cyanamid and sodium nitrate on wheat in small plots on various soil types*  
Each test in duplicate

SOIL TREATMENT*	STRAW		GRAIN		STRAW AND GRAIN	
	Dry weight	Nitrogen content	Dry weight	Nitrogen content	Nitrogen	
					Total	Recovery
	gm.	gm.	gm.	gm.	gm	per cent
<i>Portsmouth Loam</i>						
No nitrogen.....	469	1 71	213	3 99	5 70	....
Calcium cyanamid.....	514	2.24	236	5 03	7.27	31 1
Sodium nitrate.....	478	2 15	212	4 45	6 10	18 3
<i>Penn Silt Loam</i>						
No nitrogen.....	428	1.63	183	3 78	5 41	...
Calcium cyanamid.....	517	2.56	226	5.24	7.80	46.6
Sodium nitrate.....	507	2 28	198	4 70	6 98	30 9
<i>Sassafras Loam</i>						
No nitrogen.....	400	1 04	205	3.14	4.18	....
Calcium cyanamid.....	620	2.27	295	5.16	7.43	64 0
Sodium nitrate.....	615	2.15	262	4 58	6.73	50.2
<i>Sassafras Silt Loam</i>						
No nitrogen.....	177	0.58	80	1.26	1 84	....
Calcium cyanamid.....	432	1.59	166	2 92	4 51	50 4
Sodium nitrate.....	469	1.46	199	3 39	4 85	58.0
<i>Sassafras Sand</i>						
No nitrogen.....	182	0.59	92	1 47	2 06	....
Calcium cyanamid.....	344	1.03	172	3.08	4.11	40.3
Sodium nitrate.....	439	1.20	209	3.38	4.58	49 6
<i>Average of all plots</i>						
No nitrogen.....	331	1.11	161	2.73	3.84	....
Calcium cyanamid.....	485	1.93	218	4.28	6.21	46.5
Sodium nitrate.....	501	1.84	215	4 09	5.93	41.4

\* Each plot received 640 pounds superphosphate and 320 pounds muriate of potash per acre in the fall. Nitrogen added at rate of 54.2 pounds per acre, one-third in fall a week before planting, and two-thirds as a top-dressing in late March. Each treatment was duplicated.

*Comparison of effects of various nitrogen carriers on wheat grown in Latin-square field plots*

A field was divided into 25 plots by the Latin-square method. Each plot measured 4 by 6 yards. A 2-foot buffer strip of soil separated each of the plots from its neighbor. Each nitrogen material was used on five plots which were arranged in such a way that no treatment ever appeared more than once in any row of five plots in either direction.

The nitrogenous fertilizers were applied at the rate of 55 pounds of nitrogen per acre. One-third of this amount was applied at the time of planting and the other two-thirds used as a top-dressing early in the spring.

The results shown in table 3 give evidence that there is little or no difference in the availability of the nitrogen of the various materials used. Urea gave a slightly lower recovery of nitrogen, but the difference is within the limits of experimental error.

TABLE 3

*Comparison of effects of various nitrogen carriers on wheat grown in Latin-square field plots\**

SOIL TREATMENT†	STRAW		GRAIN		STRAW AND GRAIN	
	Dry weight	Nitrogen	Dry weight	Nitrogen	Dry weight	Nitrogen
	kgm.	gm.	kgm.	gm.	kgm.	gm.
Check.....	8.40	19.1	4 67	82.6	13 07	101 7
Calcium cyanamid....	9 86	31.7	5.51	109 2	15 37	140 9
Urea.....	10.20	31 2	5.14	100 3	15 34	131 5
Ammonium sulfate.....	10.40	28 5	5 15	113 2	15 55	141 7
Sodium nitrate..	10.26	30 8	5 34	109 4	15 60	140 2

\* Average of five plots of each.

† Nitrogen applied at rate of 55 pounds of the element per acre.

*Comparison of effect of various nitrogen carriers (in cylinders)*

The cylinders used in these experiments are the type of those which have long been in use at the New Jersey station (10).

*Series 1.* The surface soil selected for this series of 20 cylinders was collected from a field on the college farm and was classified as Sassafras loam. A quantity of soil sufficient to supply each cylinder with 175 pounds on the air-dry basis was sifted and thoroughly mixed before being distributed in the cylinders.

Two crops per year, a late summer crop followed by rye or wheat, were harvested from each cylinder. The nitrogen fertilizers for the winter crops were applied each year, about the middle of October, in order to determine indirectly the relative amount of nitrogen lost by winter leaching from each source of fertilizer.

The tests indicate (table 4—winter crops) that if nitrogenous fertilizers



other than the nitrates are applied late in the fall to a heavy soil on which winter grain is planted, there may be only moderate losses of nitrogen by leach-

TABLE 4  
*Crop yields and nitrogen recovery in cylinders*  
Each figure represents average of two cylinders

SOIL TREATMENT *	SUMMER CROPS						WINTER CROPS				
	Corn	Buckwheat	Oats	Corn	Total yields	Nitrogen recovery	Wheat	Rye	Rye	Total yields	Nitrogen recovery
	gm.	gm.	gm.	gm.	gm.	per cent	gm.	gm.	gm.	gm.	per cent
<i>Sassafras Loam</i>											
	Dry weight						Dry weight				
No nitrogen . . . . .	75	95	39	39	248	..	77	35	57	169	.
Sodium nitrate . . . . .	124	149	68	128	469	.	96	52	80	228	.
Ammonium sulfate . . . . .	131	146	72	119	468	.	126	105	93	324	.
Calcium cyanamid . . . . .	141	141	84	122	488	.	138	131	120	389	.
	Nitrogen content						Nitrogen content				
No nitrogen . . . . .	0 70	1 30	0 59	0 30	2 89	..	0 53	0 42	0 32	1 27	.
Sodium nitrate . . . . .	1 50	2 59	1 47	1 01	6 57	71 88	0 63	0 39	0 44	1 46	4 95
Ammonium sulfate . . . . .	1 33	2 63	1 38	0 91	6 25	65 62	0 89	0 91	0 55	2 35	28 13
Calcium cyanamid . . . . .	1 52	2 34	1 57	0 89	6 32	66 99	0 94	1 00	0 63	2 57	33 84
<i>Sassafras Sand</i>											
	Dry weight						Dry weight				
No nitrogen . . . . .	74	..	..	41	115	..	55	33	54	142	.
Sodium nitrate . . . . .	71	..	..	143	214	..	70	32	59	161	..
Ammonium sulfate . . . . .	68	..	..	138	206	..	66	48	56	170	..
Calcium cyanamid . . . . .	83	..	..	132	215	..	96	105	94	295	..
	Nitrogen content						Nitrogen content				
No nitrogen . . . . .	0 79	..	..	0 29	1 08	..	0 37	0 22	0 37	0 96	..
Sodium nitrate . . . . .	1 14	..	..	1 00	2 14	41 40	0 45	0 22	0 37	1 04	2 08
Ammonium sulfate . . . . .	1 09	..	..	1 07	2 16	42 19	0 43	0 33	0 34	1 10	3 65
Calcium cyanamid . . . . .	1 18	..	..	0 80	1 98	35 16	0 68	0 76	0 59	2 03	27 86

\* Each cylinder received the equivalent of 640 pounds superphosphate and 320 pounds muriate of potash per acre, at the time nitrogen fertilizers (supplying 41 pounds of nitrogen per acre) were applied.

ing. This is due to the fact that the ammonium ion is held firmly by the base-exchange complex of the soil, and must be converted to nitrite or nitrate before

it will be leached out to any extent. In this test, the nitrogen applied as sodium nitrate had little effect upon the crop growth, whereas ammonium sulfate and calcium cyanamid, especially the latter, gave a reasonably good increase in the crop growth and there was a fair percentage-recovery of the added nitrogen.

Street (21) working with lysimeters containing 20 inches of soil found that during a 12-month period, approximately 70 per cent of the nitrogen from sodium, calcium, and potassium nitrate was lost by drainage as compared to 60 per cent from ammonium sulfate, ammonium phosphate, and dried blood; 53 per cent from urea, castor pomace, and horn and hoof meal; 44 per cent from

TABLE 5

*Crop yields and nitrogen recoveries from use of varying amounts of calcium cyanamid*

SOIL TREATMENT	CORN*	BUCK-WHEAT*	CORN†	CORN†	AVERAGE ALL CROPS	NITROGEN RECOVERY
	gm.	gm.	gm	gm.	gm.	per cent
No nitrogen . . . . .	77	56	62	60	63	
100 lbs. calcium cyanamid	138	82	123	95	109	
200 lbs. calcium cyanamid . . . .	208	100	156	131	148	
300 lbs. calcium cyanamid	247	115	202	168	184	
400 lbs. calcium cyanamid . .	303	120	238	205	216	

*Yield of nitrogen (summer crops)*

	0 578	0 805	0 477	0 472	0 583	
No nitrogen . . . . .	0 578	0 805	0 477	0 472	0 583	
100 lbs. calcium cyanamid	0 918	1 118	0 802	0 781	0 904	46 01
200 lbs. calcium cyanamid. .	1 431	1 713	1 043	1 062	1 312	52 72
300 lbs. calcium cyanamid. .	1 696	2 179	1 590	1 322	1 696	53 76
400 lbs. calcium cyanamid . .	2 181	2 462	2 046	1 969	2 164	57 34

\* 1931. † 1932.

animal tankage, fish, linseed meal, and cottonseed meal; and 30 per cent from calcium cyanamid and cow manure.

*Series 2.* The surface soil selected for this series of cylinders was an acid Sassafra sand. One hundred grams of ground limestone was added in order to bring the pH to near neutrality. In all other respects series 1 and 2 were identical.

Data in table 4 (summer crops) show no great difference in the crop yields produced by the various fertilizers.

The winter crop yields (table 4) indicate that little of the calcium cyanamid nitrogen was lost, even from a sand, where the fertilizer was applied about the middle of October. The greater retention of the nitrogen of calcium cyanamid is no doubt due to the relatively slow nitrification which has been reported (6).

*Series 3.* A third series of cylinder tests was carried out in order to determine whether the nitrogen from large applications of calcium cyanamid was

used as efficiently by plants as that from small applications. During the fall, winter, and spring months, a crop of rye was grown in order to determine the relation between the rate of application and the amount of nitrogen recovered in the crop.

A Penn loam soil was selected for this test. The nitrogen treatment ranged from no nitrogen to the equivalent of 400 pounds of calcium cyanamid per acre.

The data given in table 5 indicate an increasing percentage recovery of the nitrogen as the rate of application increases from 100 to 400 pounds per acre.

When heavy applications of calcium cyanamid are made to soil, nitrification is retarded and may be almost entirely stopped for some time. In the case of fall applications no nitrates may be produced until the following spring. On the other hand, if the application is small and the calcium cyanamid is thoroughly mixed with the soil (table 6), the nitrification processes may be retarded very little and considerable nitrogen may be leached out. Accordingly, fall applications, if small, should not be made until after the soil temperature has

TABLE 6

*Crop yields and nitrogen recoveries from use of calcium cyanamid applied in different amounts\**  
Winter crops

AMOUNT APPLIED PER ACRE	DATE OF APPLICATION	DRY WEIGHT	NITROGEN	
			Content	Recovery
<i>lbs.</i>		<i>gm.</i>	<i>gm.</i>	<i>per cent</i>
0	.....	80	0.61	.....
200	October 8	116	0.91	21.45
300	October 8	125	1.20	28.34
400	October 8	166	1.65	37.45

\* Crops planted October 15.

fallen to the point at which nitrification is relatively slow. Fall applications should not be delayed until the ground freezes, since under such conditions satisfactory results are not obtained. The reason for this is not entirely clear. No difficulty is experienced if the calcium cyanamid is applied in the fall before the soil freezes or if it is applied in the spring after the frost is largely out of the ground.

Cowie (5) has shown that the conversion of the nitrogen of calcium cyanamid to urea is not dependent upon microorganisms, but may be accelerated by various catalytic agents present in the soil. Most soils contain an abundance of urea organisms which convert urea to ammonia even under conditions far removed from the optimum as measured by nitrate formation. Therefore, there is little danger of the nitrogen being leached out as cyanamide or urea.

#### *Comparison of various nitrogenous fertilizers in an acid sand*

Mooers (13) has claimed that calcium cyanamid is not suitable for use in an acid sand. The difficulty is probably to be attributed to the low buffer ca-

capacity of the sand. Under such conditions dicyandiamid may be formed if the calcium cyanamid is not mixed thoroughly with the soil.

To make a test of this matter, 12 one-gallon, glazed earthenware pots were filled with an equal weight of an acid Sassafras sand. The nitrogenous treatments were made at the rate of 160 mgm. of nitrogen per pot. The results given in table 7 are the average of each treatment run in triplicate. According to the results of this test, sodium nitrate is slightly superior to calcium cyanamid in a very acid sand. This is to be expected since the nitrate ion is more readily assimilated in an acid medium, as explained in the discussion. On the other hand, calcium cyanamid produced a much greater yield than did the physiologically acid fertilizer, ammonium sulfate.

*Comparison of calcium cyanamid and sodium nitrate in a sand deficient in potassium*

The purpose of this experiment was to determine whether in comparisons of calcium cyanamid and sodium nitrate the latter, by reason of its content of

TABLE 7  
*Effect of various nitrogen fertilizers on barley in an acid sand*

TREATMENT	SOIL AT END OF TEST	DRY WEIGHT	NITROGEN	
			Content	Recovery
	pH	gm.	mgm.	per cent
No nitrogen . . . . .	5.2	1.5	23 7	....
Ammonium sulfate . . . . .	4.6	1.5	54 0	18.9
Calcium cyanamid . . . . .	5.5	5.7	139 9	66.5
Sodium nitrate . . . . .	5.4	7.1	146 7	76.8

sodium, might have an advantage when there is a deficiency of available potassium in the soil.

The soil selected was a Sassafras sand which contained very little nutrient material. Two-gallon glazed pots were used. Two grams of superphosphate was applied to each pot before each planting of corn in order that phosphate might not be a limiting factor in the growth of the plants. Calcium cyanamid and sodium nitrate were applied at rates necessary to supply 320 mgm. of nitrogen per pot for each crop. In the cases where muriate of potash and sodium chloride were added, the rate was 1 gm. of each per pot.

It is evident from the data shown in table 8 that the sodium of the sodium nitrate was of considerable value under conditions of a deficiency of potassium. The sodium served only partially as a substitute for potassium, as is apparent from the better results obtained by the use of muriate of potash.

Hartwell and Damon (9) have shown that where there is an insufficiency of potassium, sodium is generally useful. This benefit is believed to be partly indirect, as by the replacement of potassium by sodium in the soil complex,

and partly in its direct effect on the plant. In other words, sodium apparently substitutes in part for potassium in the growth processes of the plant. It is conceivable that in some cases investigators who have reported a higher efficiency of the nitrogen in sodium nitrate have been misled in their interpretations on account of not having taken into consideration the sodium of the nitrate of soda.

*Comparison of the availability of the nitrogen of sodium nitrate, calcium cyanamid, dicyandiamid, and mixtures of the last two*

The purpose of the experiment was to determine whether dicyandiamid nitrogen would be available if considerable time was allowed for it to be converted to other nitrogenous compounds.

TABLE 8

*Comparison of calcium cyanamid and sodium nitrate on corn in a potassium-deficient sand*

NUMBER	SOIL TREATMENT*	SERIES	DRY WEIGHT OF CROPS		
			Crop 1	Crop 2	Crop 3
			gm.	gm.	gm.
1	Calcium cyanamid No potash	a	9 1	13 5	5 2
		b	9 8	14 6	(Lost)
2	Sodium nitrate No potash	a	14 2	22 0	6 8
		b	14 5	23 5	8 1
3	Calcium cyanamid Sodium chloride	a	13 1	23 8	6 4
		b	11 0	21 7	6 2
4	Sodium nitrate Sodium chloride	a	11 8	19 0	6 6
		b	13 2	22 0	5 1
5	Calcium cyanamid Muriate of potash	a	22 6	37 0	20 0
		b	23 5	36 0	22 2
6	Sodium nitrate Muriate of potash	a	21 5	41 2	20 1
		b	21 2	42 7	20 7

\* Superphosphate applied in all cases.

The tests were conducted in glazed earthenware pots in the greenhouse. Corn was grown in a Sassafras loam soil and also in a Sassafras sand in order to determine whether the nitrogen of dicyandiamid is more available in a heavy than in a light soil. The pH of both soils was between 6 and 7, at the beginning of the experiment. The nitrogenous materials were added at the rate of 330 mgm. of nitrogen per pot. The mixtures are listed in table 9. Only one application of nitrogen was made at the beginning of the experiment, but 2 gm. of superphosphate and 1 gm. of muriate of potash were added before each of the four successive crops in order that the lack of available phosphoric acid and potash might not become a limiting factor.

The results of the experiment reported in table 9 show that dicyandiamid, when used in sand or in an ordinary loam soil, is a slowly available source of nitrogen. As suggested by Richardson (19) the slow availability makes di-

TABLE 9

*Efficiency of nitrogen of calcium cyanamid and dicyandiamid when used on four successive crops of corn*

SOIL TREATMENT*	LOAM SOIL					SAND SOIL				
	Crop number									
	1	2	3	4	Total	1	2	3	4	Total

Dry weight										
	gm	gm	gm.	gm.	gm	gm	gm.	gm.	gm	gm.
No nitrogen . . . . .	5 0	4 3	5 2	8 2	22 7	6 9	4 9	4 9	10 7	27 4
Sodium nitrate . . . . .	7 4	7 8	7 0	9 9	32 1	8 1	8 1	6 9	12 0	35 1
All cal. cyanamid† 0 Dicy.‡ . . . .	8 0	7 0	6 4	9 6	31 0	7 8	5 8	6 6	12 1	32 3
$\frac{3}{4}$ " " $\frac{1}{4}$ " . . . . .	6 5	6 1	9 1	9 4	31.1	7 9	6 0	5 9	11 4	31.2
$\frac{1}{2}$ " " $\frac{1}{2}$ " . . . . .	6 9	5 3	10 1	9 4	31.7	7 6	6 1	6 0	11 8	31 5
$\frac{1}{4}$ " " $\frac{3}{4}$ " . . . . .	5 4	6 0	10 5	10 5	32 4	7 0	5 3	5 5	10 9	28 7
0 " " all " . . . . .	5 1	4 0	12 6	10 0	31 7	6 3	5 0	5 6	11 3	28 2

Nitrogen content										
	mg	mg	mg	mg	mg.	mg.	mg	mg	mg	mg.
No nitrogen . . . . .	93	67	50	62	272	167	100	58	92	417
Sodium nitrate . . . . .	250	137	69	74	530	270	192	79	95	636
All cal. cyanamid† 0 Dicy.‡ . . . .	253	123	60	75	511	294	190	95	96	675
$\frac{3}{4}$ " " $\frac{1}{4}$ " . . . . .	191	162	96	74	523	279	134	73	94	580
$\frac{1}{2}$ " " $\frac{1}{2}$ " . . . . .	198	119	102	71	490	274	123	73	95	565
$\frac{1}{4}$ " " $\frac{3}{4}$ " . . . . .	165	155	135	77	532	264	110	66	89	529
0 " " all " . . . . .	156	94	152	75	477	248	111	66	97	522

Nitrogen recovery										
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Sodium nitrate. . . . .	47 5	21 2	5 7	3 7	78 1	31 5	27 9	6 3	0 8	66 5
All cal. cyanamid† 0 Dicy.‡ . . . .	48 5	16 8	3 0	4 1	72 4	38 5	27 2	9 6	1 1	76 4
$\frac{3}{4}$ " " $\frac{1}{4}$ " . . . . .	29 6	28 7	13 9	3 7	75 9	34.2	10 4	4 5	0 4	49 5
$\frac{1}{2}$ " " $\frac{1}{2}$ " . . . . .	31 9	15 5	15 7	2 9	66 0	32 4	7 1	4 7	0 7	44 9
$\frac{1}{4}$ " " $\frac{3}{4}$ " . . . . .	21 8	26 7	25 8	4 4	78 7	29 4	3 2	2 5	—	93 4
0 " " all " . . . . .	18 9	8 0	30 9	4 1	61 9	24 7	3 5	2 6	1 2	32 0

\* Nitrogen materials added at the rate of 330 mgm. of nitrogen per 2-gallon pot.

† Calcium cyanamid.

‡ Dicyandiamid.

cyandiamid an unfavorable source of nitrogen for plants having a short growing season, but there may possibly be some advantages to its use in mixtures with other sources of nitrogen that are to be used for winter grain or grassland fer-

tilization. That most of the nitrogen in dicyandiamid is finally available in certain soils is proved by the data (table 9).

Doldi (7) claims that the nitrogen of dicyandiamid is as readily available as that in sodium nitrate in a soil containing 36 per cent organic matter, but that its availability decreases with a decrease in the organic matter of the soil in which it is used. Murata (14) found that ammonification of dicyandiamid was rapid under water-logged paddy-field conditions, and that it is an excellent source of nitrogen for rice.

Allison, Skinner, and Reid (2) found that dicyandiamid was actually injurious to some plants, but did not cause any definite injury other than tip

TABLE 10  
*Yields and content of nitrogen in crops from use of various forms of calcium cyanamid*

TREATMENT NUMBER	CALCIUM CYANAMID	RAPE		BUCKWHEAT	
		Dry weight	Nitrogen content	Dry weight	Nitrogen content
		gm.	mgm.	gm.	mgm.
1	None	6 93	125.1	11 03	151 7
2	Pulverized	15.83	379.3	11 87	304 2
3	Granular (composite sample)	14 93	403.3	10 77	295 2
4	Granular through 20 on 30 mesh	16.07	368 3	11 63	310 5
5	Granular through 12 on 14 mesh	15 53	368 1	11 03	285 1
6	Granular through 10 on 12 mesh	14 37	363.7	10 80	287 5
7	Granular through 8 on 10 mesh	13 43	349 6	10 47	277 2
8	Granular* 90 per cent nitrogen ration	15.13	355 5	11 20	295 1
9	Granular* 90 per cent dicyandiamid 10 per cent	15.10	391.9	9.83	274 8
10	Granular* 95 per cent nitrogen ration	15 70	369.7	11.57	299 1
11	Granular* 95 per cent dicyandiamid 5 per cent	15 00	361 4	9 37	283.1
12	Granular* 97½ per cent nitrogen ration	15.40	361.8	11.30	289.5
13	Granular* 97½ per cent dicyandiamid 2½ per cent	15.13	371.2	11.23	315.4

\* Through 10-mesh screen.

burning to corn. The results reported in this paper show almost as great recovery of nitrogen from dicyandiamid over a 5-month period, when used on a loam soil, as was obtained from sodium nitrate and calcium cyanamid. The recovery was not as great for the dicyandiamid in the case of the sand.

*Comparison of availability of the nitrogen of pulverized and granular calcium cyanamid*

The granular calcium cyanamid used in these experiments contained, in addition to cyanamide nitrogen, approximately 1.25 per cent of nitrate nitrogen. The percentages of particles (commercial granular calcium cyanamid) passing through the 12, 14, 20, 30, 40, 60, and 80-mesh screens were 99, 91, 60, 24, 11, 3, and 0.4 respectively.

This experiment was conducted in the greenhouse. The soil used was a Sassafra loam with a pH of 6.3. Rape and buckwheat were used as the test crops. Nitrogen was supplied to the extent of 330 mgm. per pot, except in the case of treatments 8, 10, and 12 (table 10) where only 90, 95, and 97½ per cent of the full ration was used, respectively.

Three of the treatments (9, 11, and 13) contained part of their nitrogen in the form of C. P. dicyandiamid. Interest in this material arises from the fact that if calcium cyanamid is applied to the soils in large granules, or in heaps, there is a tendency for part of the nitrogen to be changed to dicyandiamid rather than to urea, as the calcium cyanamid reacts with the moist soil.

The conversion of calcium cyanamid to urea is catalyzed by certain minerals in the soil. It is most rapid at a pH of 7 or lower. If the granules are large, or if the material is applied in piles and not mixed with the soil, a high alkalinity develops which favors dicyandiamid formation in the interior of the granule or group of particles, at points out of direct contact with the soil. The alkalinity is due largely to the calcium hydroxide liberated by the action of the moist soil on calcium cyanamid. It is increased by the formation of ammonia as the decomposition proceeds.

At the beginning of the experiment, each pot was weighed and the moisture content of the soil was brought up to the optimum. Each day thereafter six pots were weighed, the average loss of water was determined, and that quantity of water added to each pot. At the end of each week all the pots were weighed and any discrepancies in the moisture content were corrected.

The results of this experiment indicate that it is not advisable to have the calcium cyanamid granules too large. The use of the smaller sized separates of granular calcium cyanamid results in crop yields and nitrogen recoveries equal to those obtained from the pulverized calcium cyanamid. When the larger granules were employed crop yields and nitrogen recoveries were somewhat lower.

The plants receiving treatments, 6, 7, and 9, especially the last two, showed some tip-burning. This is characteristic of plants fertilized with dicyandiamid. This material is relatively stable in the soil, its nitrogen becoming available to the plant very slowly.

#### *Comparative effect of nitrogen carriers on soil reaction*

The primary purpose of this experiment was to determine the effect of various nitrogen carriers on the reaction of the soil:

- In the presence of a crop, soil not leached.
- In the presence of a crop, soil leached.
- In the absence of a crop, soil not leached.
- In the absence of a crop, soil leached.

The secondary purpose was to determine the effect of the fertilizer treatments on the crops grown.



A Sassafras loam soil was well mixed and screened. Sixteen-pound portions were then placed into each of 52 two-gallon glazed coffee urns. The holes in the bottom of 26 of the urns were closed with rubber stoppers. One-hole rubber stoppers, fitted with glass tubing which led to individual 2½-liter bottles, were placed in the openings of the other 26 urns.

Five crops of barley and five crops of corn were grown in each pot. Each pot received the equivalent of 50 pounds of  $K_2O$ , 60 pounds of  $P_2O_5$  (with the exception of No. 11 which received extra phosphoric acid), and 50 pounds of nitrogen per acre (with the exception of No. 1), before each crop was planted. At the end of the experiment, 500 pounds of  $K_2O$ , 500 pounds of nitrogen, and 600 pounds of  $P_2O_5$  (on the acre basis) had been added per pot. All the  $K_2O$  was applied as muriate of potash.

Seven and one-half liters of water were passed through each leached soil on which a crop was grown. Five liters were passed through each leached soil where no crop was grown.

The differences in the crop yields produced by the several fertilizer applications (table 11) are not important, being probably within the limits of experimental error.

When calcium cyanamid or nitrate of soda was used in conjunction with orthophosphoric acid or ammonium phosphate, the net effect was a marked rise in the pH of the soil. A somewhat smaller rise in pH occurred in the case of the calcium nitrate treatments. All other treatments resulted in a lowering of the pH. This was especially noticeable following the use of ammonium sulfate. The test indicates that it is possible to balance the acid effects of orthophosphoric acid and ammonium phosphate by the supplemental use of alkaline carriers of nitrogen such as calcium cyanamid, sodium nitrate, and nitrate of lime.

Very little difference was noted between the effects of sodium nitrate and calcium cyanamid on the pH of the soil, most likely because the Sassafras loam used in this experiment was fairly well buffered. Fudge (8) and Pierre (15), however, have found that calcium cyanamid causes a greater decrease in the acidity of the soil than does sodium nitrate. The results of the unsaturation test (table 11) tend to confirm their results.

#### *Effect of various nitrogenous fertilizers and hydrated lime on the pH of the soil*

A Sassafras loam soil with a pH of 5.4 was used to determine the effect of the various materials on the reaction of the soil. The tests were carried out in 1-gallon glazed earthenware pots.

Calcium cyanamid and C. P. hydrated lime were each applied at the rate of 1,000 pounds per acre. The other nitrogen materials were applied in such amounts as to add the same quantity of nitrogen as that supplied by calcium cyanamid. All materials were thoroughly mixed with the soil.

The soil moisture was kept as near the optimum for the particular soil as was possible. After 60 days, corn was seeded in each pot in order to remove excess

TABLE 11  
*Effect of various nitrogen fertilizers upon growth and nitrogen content of crop and pH of soil*

NUMBER	SOIL TREATMENT	SOIL NOT LEACHED				SOIL LEACHED			
		Dry† weight	Nitrogen content	pH of soil		Dry† weight	Nitrogen content	pH of soil	
				Cropped	Not cropped			Cropped	Not cropped
1	No nitrogen. Phosphoric acid*	44.2	1,056	6.2	5.9	42.4	1,021	6.3	6.3
2	Calcium cyanamid plus phosphoric acid	69.6	2,761	6.9	6.1	63.2	2,477	6.9	6.5
3	Sodium nitrate plus phosphoric acid	65.9	2,827	7.0	6.1	58.3	2,127	6.8	6.4
4	Calcium nitrate plus phosphoric acid	68.6	2,730	6.6	6.0	57.5	2,047	6.5	6.3
5	Ammonium sulfate plus phosphoric acid	73.0	2,868	5.2	4.6	60.6	2,211	5.2	4.7
6	Nitric acid plus phosphoric acid	71.9	2,876	6.0	5.1	62.9	2,232	6.0	5.5
7	Ammonium hydroxide plus phosphoric acid	70.6	2,866	5.5	5.1	65.6	2,499	5.2	5.1
8	Ammonium hydroxide plus phosphoric acid and gypsum	70.1	2,923	5.4	5.1	63.1	2,429	5.3	5.1
9	Ammonium hydroxide plus superphosphate	68.4	2,811	5.9	5.3	66.2	2,503	6.0	5.2
10	Ammonium sulfate plus superphosphate	69.9	2,726	5.2	4.7	59.3	2,140	5.1	4.8
11	Ammonium phosphate (commercial 11-48-0)	71.1	2,815	5.4	5.1	61.6	2,211	5.4	5.2
12	Ammonium phosphate plus sodium nitrate	68.0	2,822	6.8	5.7	61.3	2,219	6.6	6.3
13	Ammonium phosphate plus ammonium sulfate	68.4	2,777	5.2	4.7	60.6	2,145	5.2	4.7

\* Orthophosphoric acid.

† Total for 10 successive crops.

nitrate from the soil. Table 12 shows the materials used, the length of time after treatment at which pH determinations were made, and the pH of the soil of each pot.

Commercial calcium cyanamid contains calcium equivalent to about 70 per cent calcium hydroxide. Most of this calcium is in readily soluble and active forms  $[\text{CaCN}_2 \text{ and } \text{Ca}(\text{OH})_2]$ . When the  $\text{CaCN}_2$  comes in contact with moist soil, it is rapidly broken down into urea and calcium hydroxide. The urea is rapidly converted to ammonia, which in turn is slowly converted to nitrate. While the nitrogen is in the form of ammonia, it aids the hydrated lime in raising the pH of the soil.

The net result of these various changes upon the pH of the soil is clearly shown in table 12. Calcium cyanamid caused the pH to rise considerably above that

TABLE 12

*Effect of various nitrogen materials and of hydrated lime on the pH of Sassafras loam soil*  
Original pH 5.4

TREATMENT	pH AT FOLLOWING PERIODS AFTER TREATMENT								
	4 hours	1 day	4 days	7 days	11 days	16 days	32 days	60 days	After crop- ping corn
	pH	pH	pH	pH	pH	pH	pH	pH	pH
Calcium cyanamid*.....	6.5	6.6	6.8	6.6	6.6	6.5	5.7	5.3	5.7
Ammonium sulfate.....	5.4	5.3	5.1	5.0	5.0	5.0	4.5	4.4	4.9
Urea.....	5.6	6.1	6.1	5.9	5.3	5.2	4.6	4.6	5.1
Hydrated lime*.....	6.5	6.4	6.4	6.4	6.3	6.3	6.3	6.3	6.3
Urea and hydrated lime*.....	6.6	6.9	6.8	6.3	5.9	5.6	5.5	5.5	5.9
Ammonium sulfate and lime*.....	6.5	6.4	6.1	5.9	5.6	5.2	4.9	4.8	5.5

\* 1,000 pounds per acre. Nitrogen materials applied in such amounts to give equal quantities of the element.

produced by an equal weight of hydrated lime, and then it slowly dropped to a value slightly below that of the soil before treatment. When a crop had been grown on the soil, the pH again rose somewhat above that of the original soil. In other words, the net effect of the calcium cyanamid on cropped soil was to raise its pH. Ammonium sulfate and urea, particularly the former, lowered the pH.

*Effects of continuous use of various nitrogen materials on physical and chemical properties of the soil*

After the eleventh crop was harvested from the pots used in the experiment on the effect of the continued use of the various nitrogenous fertilizers on the crop yield (table 1), the soil from each pot was thoroughly mixed and passed through a 2-mm. sieve, after which it was used for the following studies:

A portion of soil from each pot was used for a percolation experiment. Five

percolation tubes, 2 inches in diameter and deep enough to allow the addition of 12 inches of soil were set up. The same weight of each of the five soils was placed in the tubes. The water was applied in each tube. When the water began to percolate through the bottom of the tubes it was turned off, and the tubes were allowed to stand for 1 day. A constant flow of water was then sent through the top of each of the tubes, and the amount of water percolating through the soil in 16 hours was measured. The results are shown in table 13.

A second portion of each soil was taken to test the rate of settling of the soil colloids. Twenty-five gram portions of each of the soils were placed into calibrated graduates. To each graduate was added 100 cc. of distilled water. Each mixture of soil and water was then shaken for 2 minutes, and allowed to stand for 20 minutes. The photograph (pl. 1, fig. 2 a) was then taken of the five soils. Another photograph (pl. 1, fig. 2 b) was taken after 3 days' standing.

Another portion of each of the aforementioned soils was taken for pH determinations. The active acidity of each soil was determined by the potentio-

TABLE 13  
*Effect of nitrogen carriers on rate of percolation of water through soil*

SOIL TREATMENT	PERCOLATE
	cc.
No nitrogen.....	405
Sodium nitrate.....	48
Ammonium sulfate.....	1,286
Calcium cyanamid.....	510
Tankage.....	1,248

metric method. The exchange acidity was determined by the use of indicators in a mixture of 1 part of soil and 2 parts of  $N Na_2SO_4$ .

A fourth portion of each of the five soils was analyzed for its base-exchange capacity and the percentage unsaturation at pH 7. Twenty grams of each soil was leached with neutral barium acetate until the pH of the leachate became neutral. The soil was then treated with a small amount of barium chloride solution and washed with water until the leachate gave no test for chlorine. This was done to insure the removal of all free barium not held by the base exchange complex.

The leachate from the barium acetate treatment was titrated against 0.1  $N$  sodium hydroxide, and the milliequivalents of hydrogen per 100 gm. of soil calculated.

The total base-exchange capacity at pH 7 was then obtained by removing the barium from the soil by treatment with 0.1  $N$  ammonium chloride which was continued until small portions of the leachate gave no precipitate when sulfuric acid was added. The number of milliequivalents of barium was then calculated for 100 gm. of soil by precipitating the barium as the sulfate. The per cent unsaturation was calculated (table 14).

The results of these experiments show that the several nitrogen materials employed in these tests are markedly different in their effects on the physical and chemical properties of the soil.

In the percolation and rate-of-settling studies, ammonium sulfate and tankage were outstanding in their effects. Water percolated through the soil treated with calcium cyanamid more rapidly than through the untreated soil. Their action was in marked contrast to that of sodium nitrate. The soil treated with sodium nitrate became very hard after wetting and being allowed to dry out. This undesirable physical condition caused the crop yields to fall as compared with those produced by calcium cyanamid and tankage.

In the base-exchange and pH studies, the definitely acid effect of the ammonium sulfate was quite apparent, as compared to the alkaline effects of sodium nitrate and calcium cyanamid. The soil treated with ammonium sulfate had dropped to pH 4.2, by the quinhydrone-electrode method, and

TABLE 14  
*Effect of nitrogen carriers on base exchange capacity and pH of soil*

SOIL TREATMENT	HYDRO- GEN	BASE EXCHANGE CAPACITY	UNSATU- RATION	pH	
				Quin- hydrone electrode	Colori- metric†
	<i>m.e.*</i>	<i>m.e.*</i>	<i>per cent</i>		
No nitrogen. . . . .	3 62	11 40	34 8	5 9	5 6
Sodium nitrate . . . . .	1 37	11 03	12 5	6 8	6 1
Ammonium sulfate . . . . .	8 50	8 94	95 0	4 2	4 3
Calcium cyanamid. . . . .	1 22	10 95	11 2	6 8	6 4
Tankage. . . . .	4.12	11 03	37 5	5.8	5 4

\* Per 100 gm. soil.

† In  $N Na_2SO_4$ .

had reached the stage of 95 per cent unsaturation at the end of the test. The yields had begun to fall by the end of the fifth successive crop.

*Effect of the pH of the soil on the availability of the nitrogen of calcium cyanamid*

An attempt was made in this experiment to determine the approximate pH at which the greatest recovery of nitrogen from calcium cyanamid takes place.

Three soils with a very low pH were selected for the test. The crops were grown in 2-gallon glazed earthenware pots. Each pot received 320 mgm. nitrogen in the form of calcium cyanamid. The pH of the soil was raised by adding increasingly larger amounts of calcium carbonate. Each treatment was run in triplicate, but, in order to conserve space, only the averages of the three pots are given in table 15. The pH readings were made after the crops were harvested.

The results of this experiment show that the nitrogen of calcium cyanamid is not used efficiently when the pH of the soil is very low. The barley crop,

which is sensitive to acid soils, gave very little growth at pH values below 5.0. It would appear from the results in table 15 that a pH of about 6.0 would have to be reached before a maximum recovery of nitrogen could be expected.

TABLE 15

*Effect of varying the pH on recovery of nitrogen from calcium cyanamid*

pH OF SOIL	BARLEY			CORN		
	Dry weight	Nitrogen content		Dry weight	Nitrogen content	
		gm.	per cent		gm	per cent
<i>On Sassafras sand</i>						
4.9	1.57	1.59	25	4.73	1.61	76
5.5	10.37	2.25	233	13.63	1.91	261
6.4	12.67	2.08	263	15.75	2.00	311
6.8	13.83	2.08	287	17.87	1.87	335
7.1	12.27	2.17	266	16.70	1.91	319
7.3	11.35	2.23	253	15.60	2.07	323
7.5	11.45	2.28	261	13.67	1.87	265
7.6	12.00	2.28	273	12.65	2.02	256
<i>On Sassafras loam</i>						
5.0	6.00	3.17	190	11.00	2.74	301
5.1	10.60	3.71	393	11.50	3.18	366
5.6	14.93	3.55	530	10.93	3.28	358
6.0	15.23	3.73	568	11.47	2.82	323
6.6	16.23	3.97	645	8.25	3.03	250
7.0	15.63	4.18	654	9.60	2.89	277
7.3	15.63	4.41	690	8.07	3.30	266
7.4	15.70	4.28	672	8.17	3.30	270
<i>On Penn silt loam</i>						
4.6	0.70	1.14	8	4.25	1.60	68
4.7	1.20	1.67	20	7.57	2.26	171
5.1	3.43	3.09	106	10.37	2.61	271
5.4	7.77	2.60	202	11.80	2.70	319
5.7	8.60	2.81	242	9.87	2.59	256
6.1	8.93	3.16	282	8.77	2.85	250
6.3	9.33	2.93	274	10.23	2.73	279
6.7	10.60	3.00	318	9.83	2.98	293

Even at a pH considerably above the neutral point, for example 7.62, the crop growth was very near the maximum.

The corn crop is rather tolerant to soil acidity, and yet the results show that where the pH of the soil is below 5.0, the crop yields and the total nitrogen contained in the crops are low. At all pH values above 5.0 the corn plants made a good growth.

*Calcium cyanamid as an agent for weed control in timothy*

Two plots of timothy, each one-twentieth of an acre, were treated with the equivalent of 200 pounds of calcium cyanamid per acre. At the end of the growing season, the entire growth was weighed, and a representative sample of hay was taken from each plot. The different types of weeds were separated from the timothy and clover. Each separate was weighed and calculated on the acre basis.

The calcium cyanamid was applied early in the spring or just after the timothy and weeds had begun to grow. It was applied by hand early in the

TABLE 16

*Effect of calcium cyanamid\* as a control for weeds in timothy (acre basis)*

	SEASON OF 1931				SEASON OF 1932			
	Total plants	Dry weight	Nitrogen	Protein	Total plants	Dry weight	Nitrogen	Protein
	per cent	lbs.	per cent	lbs.	per cent	lbs.	per cent	lbs.
<i>Treated with calcium cyanamid</i>								
Timothy.....	70.84	3,220	0.75	108.8	87.02	1,522	1.03	98.0
Clover.....	.....	.....	.....	.....	0.34	6	2.04	0.8
Daisy.....	25.65	840	0.62	30.6	11.04	193	0.97	11.7
Miscellaneous weeds.....	3.51	115	1.42	10.2	1.60	28	1.21	2.1
Total.....	100.00	4,175	.....	149.6	100.00	1,649	...	112.6
<i>Not treated with calcium cyanamid</i>								
Timothy.....	34.55	880	0.74	40.7	76.05	508	1.08	34.3
Clover.....	1.93	49	1.99	6.1	1.79	12	2.07	1.6
Daisy.....	59.67	1,520	0.41	39.0	11.83	79	0.64	3.2
Miscellaneous weeds.....	3.85	97	1.02	6.2	10.33	69	0.86	3.7
Total.....	100.00	2,546	...	92.0	100.00	668	...	42.8

\* 200 pounds calcium cyanamid per acre.

morning while the dew was still on the plants, in order that more of the material might stick to the leaves.

These treatments would no doubt have been more effective if the calcium cyanamid had been applied with a dusting machine, which would have distributed it more evenly than is possible by a hand application.

The results of the treatments in 1931 and 1932 (table 16) both show an approximately 50 per cent control of the weeds. In each case, the yield of timothy was about three times as heavy on the treated plot as that produced on the check plot.

Bissy and Butler (3) were able to kill 80 per cent of the mustard in a grain field by applying 80 pounds of calcium cyanamid to the moist foliage. Sturkie

(22) was able to kill all the weeds on Bermuda-grass lawns with an application of 800 pounds per acre. Lighter applications were partially effective. Two thousand pounds per acre tended to injure or kill the leaves of the grass, but after a period of 2 weeks new leaves formed, and an excellent lawn was obtained.

#### DISCUSSION

Although the earlier investigations, prior to 1925, indicated that calcium cyanamid is not as available a source of nitrogen as are the common inorganic fertilizers, so considerable advancement has since been made in the knowledge of the correct methods of application that, with few exceptions, the more recent investigations have made it apparent that calcium cyanamid and the common inorganic nitrogenous fertilizers are of very nearly equal value as sources of nitrogen (11).

In an intensive investigation upon the decomposition of calcium cyanamid in the soil and its effects on germination, Crowther and Richardson (6) found that when calcium cyanamid is thoroughly mixed with the soil it is chemically converted to urea, which in turn is converted microbiologically to ammonia all within a few days. They concluded that its injurious effect on germinating seeds is caused by free cyanamid, which has a very high penetrating power and, therefore, the fertilizer should be applied some time before planting.

Buchanan and Barsky (4) made a study of the decomposition of calcium cyanamid in solution and point out that in soils of a pH below 7, dicyandiamid can not be formed from calcium cyanamid unless the latter is applied in such a manner that its alkalinity exceeds the buffering capacity of the soil.

*Methods of use.* Because of its peculiar properties, more care must be exercised in the use of calcium cyanamid than is necessary with most other nitrogenous fertilizers. The destruction of germinating seeds, which is so often mentioned, is caused by cyanamide itself, and not by its decomposition products (6). With proper methods of use, such as mixing the fertilizer with moist soil and allowing an interval of 3 days for every 100 pounds per acre between application of the fertilizer and planting of the crop this difficulty is overcome. Crowther and Richardson (6) also state that the decomposition of calcium cyanamid to urea can be accelerated by a number of minerals that may occur in the soil. Very few soils are deficient in the required catalyst, and the conversion of calcium cyanamid to ammonia is complete within a few days.

The "3 days for every 100 pounds" period is in most cases longer than necessary to overcome this effect of calcium cyanamid. In many of the preliminary tests on disease control (not all reported in this paper), the seeds were planted as soon as the fertilizer had been applied, and in no case did a 400-pound per acre application (mixed to a 6-inch depth) cause any retardation or decrease in germination. The foregoing recommendation should, however, be followed in order to be safe under conditions which are not favorable for the most rapid conversion—such as its use in a very sandy soil, or in soils with a low moisture content.



Calcium cyanamid is an alkaline material, having the property of being partially converted to dicyandiamid when it comes into contact with moisture unless an appropriate buffer, such as soil, is present to prevent the pH from rising above about 8.0. Commercial calcium cyanamid is stored and shipped in paper-lined bags in order to prevent it from coming in contact with moisture in the absence of soil. Bags should not be opened before the material is to be used, if the maximum efficiency of its nitrogen is desired.

As shown by Richardson (19), the same conversion also tends to take place when calcium cyanamid is mixed with superphosphate in such amounts as to exceed the acid content of the superphosphate.

Calcium cyanamid should not be applied in thick streams or piles, nor should it be applied as a side-dressing for vegetable crops unless it is mixed with the soil by cultivation soon after it is applied. Very heavy applications should be avoided on poorly buffered or alkaline soils. If these precautions are not taken, the alkalinity produced by the calcium cyanamid may greatly exceed the buffer capacity of the soil and some dicyandiamid, which is slowly available, may be formed. Good results are obtained by the use of calcium cyanamid as a top-dressing for pastures, but here again it should be allowed to fall on a board as it comes out of the drill, so as to spread it evenly instead of in seams. Top-dressings should be made early in the spring after the frost is out of the ground or in the fall before the ground freezes.

Calcium cyanamid should not be applied as a surface application to dry soil or to frozen soil. The soil should be moist and unfrozen so that the calcium cyanamid can diffuse into it and be subjected to action of the soil. Otherwise the calcium cyanamid, by lying on top of the dry soil and not diffusing into it, may be partly changed to dicyandiamid with consequent reduction in the availability of its nitrogen. Applications to dry surface soil should be made only if rain is imminent or if irrigation is to be practiced. In side-dressing cultivated crops, calcium cyanamid should be applied in the bottom of a newly-made furrow and mixed with the moist soil.

*Nitrogen availability.* The fact that all forms of nitrogen are not equally available for plant growth under all conditions has long been established. Because of numerous variable factors such as the reaction, type, temperature, and moisture content of the soil; the type of crop being grown; and the methods of distribution and time of application of nitrogen fertilizers, it is impossible to give any nitrogen material a definite rank as being more or less useful than another nitrogen material under all conditions.

The value placed on nitrates as sources of nitrogen has frequently been higher than that for calcium cyanamid, on the assumption that plants are more able to assimilate nitrogen in the nitrate form and that the nitrification of the nitrogen of calcium cyanamid is too slow. That this assumption is at least partially false has been proved by a number of investigations on the assimilation of the various forms of nitrogen by various plants. Tiedjens and Robbins (25) study-

ing the use of nitrate and ammonia nitrogen by certain crop plants, mainly tomatoes, soybeans, and peach seedlings, make the following statement:

Theoretically, ammonia would seem to be a more efficient source of nitrogen than nitrates, as eventually ammonia is formed from nitrates in the process of synthesis of organic nitrogen. Likewise, in actual fact, ammonia in a nutrient solution at high pH proved to be a more quickly available form of nitrogen than nitrate, as indicated by more rapid stem elongation, greater leaf area, greater succulence, darker green color, and lower carbohydrate content of the plant tissue.

Prianishnikow (17) is of the opinion that nitrification is not a desirable process from the standpoint of plant nutrition, since less energy is expended in converting ammonia into protein than in converting nitrates. He also states that ammonia enters the plant faster than nitrates, and is more easily assimilated in the synthetic process. He claims that with an excess of ammonia, injury appears more readily than with an excess of nitrates, and that nitrification is, so to speak, a hygienic function which regulates the concentration of ammonia in the soil. According to his investigations (18), ammonium gives the better results in a neutral medium while in an acid reaction, nitrate is the better source of nitrogen for plants. By adjusting the hydrogen-ion concentration as well as that of the other cations present in the nutritive medium, the nutrition of the plant can be so altered that its optimum development will result from either the use of ammonia or of nitrate.

It has been claimed (1) that calcium cyanamid is a slowly-available source of nitrogen and that it is not satisfactory for use on crops which require most of their nitrogen during the earlier stages of their growth.

The retardation reported in the growth of cotton following the use of calcium cyanamid was probably due to the high concentration of ammonia. The common method of applying fertilizer to cotton has been to drill it into the old finishing furrow.<sup>3</sup> A center furrow is run through the fertilizer to spread it out somewhat and mix it with the soil. The soil is then bedded back and seeded. The rate of application, which did not compare favorably with other sources, was 80 pounds of nitrogen in row treatments. By the use of this method, the fertilizer would be very concentrated, causing a very slow nitrification. It is conceivable that the ammonia, which is held firmly in place by the base-exchange complex of the soil, was so concentrated in the portion of the soil which contains the roots of the cotton plant that ammonia injury resulted. Since plants, especially those low in carbohydrates, are not as sensitive to nitrate nitrogen, and since nitrate nitrogen is free to move in the soil, the same retardation would probably not result from the use of a nitrate or of any form of nitrogen which is rapidly converted to nitrate. Tiedjens (24) has demonstrated that ammonia is an excellent source of nitrogen for cotton when the pH is high, but in cultures where the pH was held near 3.5 and 4.5, the ammonia produced a very scant growth.

<sup>3</sup> Furrow left between rows at last cultivation of previous crop.

The investigations of Pirschle (16) show that ammonia is effective in producing abundant crop growth when the pH of the medium is near or above neutral (above pH 6.), but that nitrate nitrogen is the better source of nitrogen under acid conditions. In this respect calcium cyanamid has the unique property of raising the pH of the soil (table 12) higher than an equal weight of hydrated lime. As nitrification nears completion the pH is again lowered to near the value of the original soil. During the time that the nitrogen is in the form of ammonia, however, this property tends to hold the pH where it is more favorable for ammonia assimilation.

The relatively slow nitrification of calcium cyanamid is of value because ammonia nitrogen is not readily leached from the soil. This should be of considerable importance on the sandier types of soil, where fall-fertilization is practiced. The common practice of side-dressing is not as necessary where the nitrogen remains in the form of ammonia for a considerable period.

*Effect upon the soil.* The larger percentage of calcium contained in calcium cyanamid causes a flocculation of the soil colloids, and has a tendency to improve the physical condition of soils high in clay. In addition, calcium cyanamid has a greater tendency to counteract soil acidity and to increase the degree of saturation with bases than have any of the common nitrogenous fertilizers (20).

*Dicyandiamid and the availability of its nitrogen.* The literature contains many references to dicyandiamid. In some cases in which calcium cyanamid produced lower yields than did other nitrogenous fertilizers with which it was compared, dicyandiamid was mentioned as a probable cause of the lower yield. Although small amounts of dicyandiamid, when mixed and applied with other nitrogenous fertilizers, do not affect the nitrogen recovery to any appreciable extent, as shown in table 10 and reported by McGuinn (12) and others, there can be little doubt that improper methods of applying calcium cyanamid may result in the production of enough dicyandiamid in the soil to reduce the effectiveness of the nitrogen, since the nitrogen of dicyandiamid becomes available to plants only very slowly. However, if calcium cyanamid is well distributed over or mixed with moist soil, little or no dicyandiamid will be formed.

Dicyandiamid is not a particularly toxic form of nitrogen (6) but, because of its stability under ordinary soil conditions, its effects continue over a longer period. Under some conditions, such as in paddy-field fertilization, dicyandiamid is a valuable source of nitrogen.

*Weed control.* Pulverized calcium cyanamid is effective in destroying weeds which have a broad leaf. If it is applied when the weeds are young and when they are moist (after a rain or heavy dew), the dust adheres to the broad hairy leaves and as they dry they are killed. For this reason, it should never be used for killing weeds in vegetable plots (with the exception of asparagus) or other broad-leaved domestic plants, as the same principle applies. On the other hand, the leaves of grass plants are narrow and usually smooth on the upper surface so that less of the calcium cyanamid will adhere. There is usually some

injury to the exposed grass or grain leaves, causing a temporary change in the color of the foliage. In very extreme cases (21), the exposed part of the leaves may even be killed, but since the leaves grow out of a protected sheath the injury is only temporary and the unexposed portion soon replaces that which was injured.

Even distribution is of major importance in the destruction of weeds.

#### SUMMARY AND CONCLUSIONS

From the results of these experiments and the discussion, a number of conclusions may be drawn:

Calcium cyanamid, properly applied, gives crop yields equal to those produced by an equal amount of nitrogen from other high-grade materials. The nitrogen recovery is on the average equal for calcium cyanamid, sodium nitrate, ammonium sulfate, and urea.

In a very acid sand (under conditions that do not allow leaching—as in pots), sodium nitrate is slightly superior to calcium cyanamid, which in turn gives a much greater yield of dry matter and recovery of nitrogen than does ammonium sulfate.

The nitrogen from calcium cyanamid does not leach from the soil as readily as does that from inorganic nitrogenous materials.

The high percentage of calcium in calcium cyanamid has a beneficial effect upon the physical properties of the soil.

Calcium cyanamid causes a greater temporary decrease in hydrogen-ion concentration than does an equal weight of C. P. calcium hydroxide. It has a permanent physiologically alkaline effect.

Pulverized calcium cyanamid may be used to destroy weeds in grassland or small grains. It should be applied in the early spring and in the morning while the dew is still on the plants. The grass or grain itself usually changes from a green to a brownish color for a very short period, after which growth is rapid as a result of the available nitrogen present and of the elimination of a large percentage of the weeds.

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## PLATE 1

FIG. 1. Sixty frames constructed from 2 inch by 12 inch boards, each frame 1 square yard in area. The frames have no bottoms and the added soil, previously screened and mixed, rests directly on the native subsoil.

FIG. 2. Settling of soil colloids. A. Twenty minutes after mixing. B. Three days after mixing. 1, No nitrogen; 2, calcium cyanamid; 3, tankage; 4, sodium nitrate; 5, ammonium sulfate.

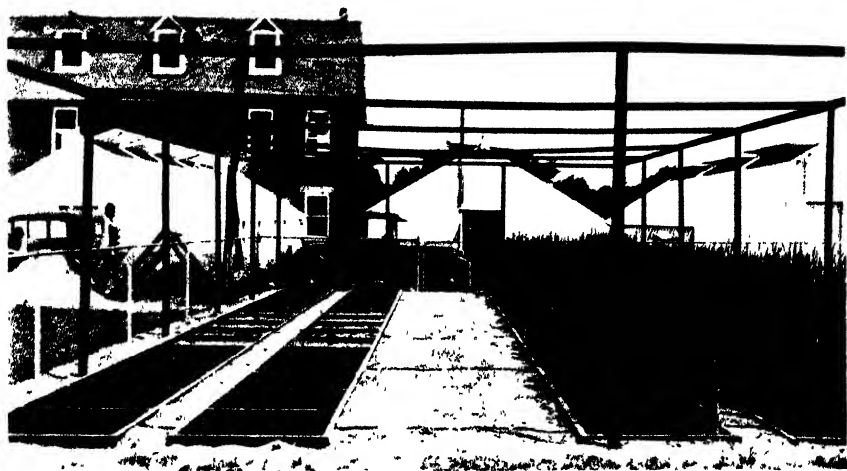


FIG. 1

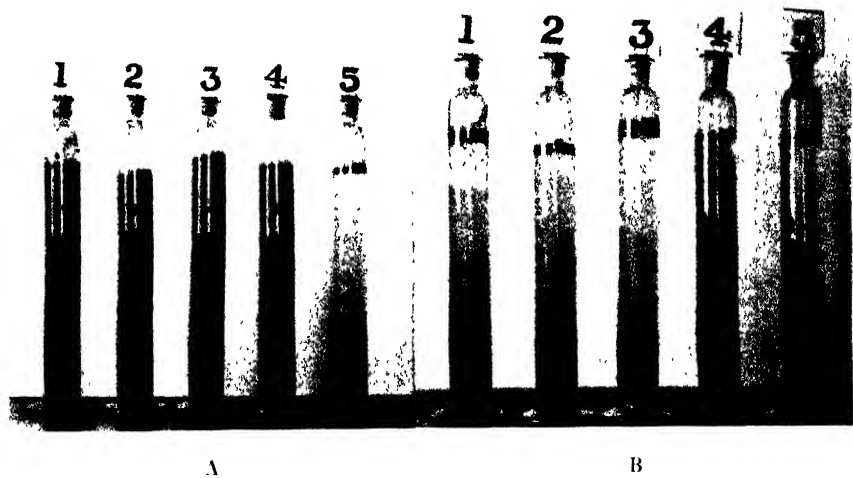


FIG. 2



# PRESERVATION OF SOILS AGAINST DEGENERATION<sup>1</sup>

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In previous reports (4, 5, 6) the writer has shown the importance of an intimate natural or artificial mixture of highly organic and nearly neutral colloidal matter in imparting high base exchange capacity, nitrogen content, and nutrient supplying power to soils.

The present study was undertaken to determine how much additions of lime, organic matter, and other soil building treatments used on some of the older experiment fields in the eastern states and in Oregon have affected the supply of total nitrogen, organic matter, and base exchange capacity of the soils of different regions. The old plat soils collected and analyzed included representatives from humid, semi-arid, and arid sections.

The work of Gedroiz (2) and that of Byers and Anderson (1) have shown striking differences in the clay fraction of the great soil groups such as are brought about by the process of podzolization under humid conditions with extensive leaching of the products of soil hydrolysis. The intensity of this process is recognized to be dependent upon the climatic conditions and related vegetation. Under tropical humid or perhaps alkaline conditions soil colloids tend to become largely decalcified or desilicated and the base adsorbing complex degenerated into simple oxides or end products with marked reduction in base exchange capacity. There is abundant evidence of a tendency to decrease soil nitrogen and organic matter by continuous cropping and cultivation in humid climates due to both increased bacterial action and alteration of the colloid complex. For example, Shutt (10) found Manitoba prairie soil nitrogen content to decrease from 0.651 to 0.506 per cent and loss on ignition to decrease from 19.43 to 14.79 per cent with 25 years of cultivation. The writer has previously noted (7) that organic matter and nitrogen can be more readily built up in calcareous soils under irrigation farming. Soil change due to long-continued cultivation has been the subject of a special committee of the American Soil Survey Association for some years and the present study was undertaken as a contribution of the writer to the work of that committee.

## EXPERIMENTAL WORK

To determine the extent to which base exchange capacity and other soil characteristics may be affected by long-continued cultural treatments, a

<sup>1</sup>Published as technical paper 201 with the approval of the director as a contribution from the soils department, Oregon Agricultural Experiment Station.



chemical study was undertaken of soil samples from the older experiment stations.<sup>2</sup> The samples were prepared and analyzed by the current methods in use in the laboratories of the Oregon station perviously described (4).

*The results of determinations with soils from Missouri, Ohio, and Illinois Station plats.* Table 1 is arranged to show the value of additions of organic matter and lime in building up soils in long-time experiments. It will be seen from the first section of the table that the Wooster silt loam from the Wooster station has been improved in nitrogen and organic matter content by the addition of lime and floats, or by manuring, particularly in the surface soil. This has been accompanied by an increase in phosphate supplying power and has resulted in a maximum base exchange content for the plats represented. A definite increase in base exchange content has resulted from manuring.

Samples from the Morrow plats at the Illinois experiment station gave results as reported in the second section of table 1. Some increase in organic matter was found, where the soil was treated with lime, manure, and phosphorus under continuous corn culture, and this increase extends to subsoil samples taken at from 19 to 30 inches. The increase with this treatment, using a 2-year rotation corn-oats, has been about 50 per cent in organic matter and is noticeable to the 30-inch level. There has been a moderate increase in total nitrogen from these treatments, and this tends to extend into the subsoil. Base exchange content appears to have been affected but moderately by these treatments. Phosphate supplying power of the soil has been greatly improved.

At the Sanborn experiment field of the Missouri station manuring and rotation treatments are included. These have given a marked increase in organic matter and total nitrogen. In some cases the manuring treatment has practically doubled the supply of these constituents as compared to untreated checks. There has been an apparent build-up in base exchange capacity from manuring and apparently from rotation treatment. Manure has increased the available phosphorus. No comparison with the virgin soil has been attempted.

*Soil changes from rotation, manure, and supplemental irrigation at Corvallis, Oregon.* Striking increases in nitrogen and organic matter have previously been reported from the first 15 years of manuring, rotation, and supplemental irrigation in the bean rotation of the irrigation experiment field at the Oregon Agricultural Experiment Station at Corvallis. The soil is Amity silty clay loam, under a sub-humid climate. Further determinations reported in table 2 show the effect of pursuing these treatments for a period of 20 years, upon nitrogen, organic matter, and base exchange capacity. There has been a definite trend toward increased nitrogen and organic matter both with and with-

<sup>2</sup> Soil samples were kindly supplied by Professors M. F. Miller, R. S. Smith, and Earl E. Barnes of the Missouri, Illinois, and Ohio State Agricultural Experiment Stations, respectively. The writer is grateful to Clarence Burnham and J. L. Wursten, graduate assistants, for assistance in making determinations herein reported. Acknowledgment is made to Dr. H. G. Byers for reading and giving helpful criticisms of this article.

TABLE 1

*Value of organic matter and lime in building up soils from long-time experiment fields—Ohio, Illinois, and Missouri Stations*

PLAT	DEPTH	TREATMENT	pH	ORGANIC MATTER	TOTAL NITROGEN	BASE EXCHANGE CONTENT	AVAILABLE PHOSPHORUS
	<i>inches</i>			<i>per cent</i>	<i>per cent</i>	<i>m.e. 100 gm</i>	<i>p.p.m.</i>
<i>Ohio Station</i>							
†14	0-7	Check	7.65	1.30	0.0896	18.04	9
14	7-12			0.65	0.0560	7.86	
14	12-20			0.57	0.0560	7.66	
†15	0-7	Manure	7.48	1.30	0.0980	27.70	9
15	7-12			1.00	0.0588	9.06	
15	12-20			0.83	0.0560	9.50	
*7	0-7	Check	6.25	1.20	0.0980	29.49	7
7	7-12	Lime accidentally		0.70	0.0420	6.84	
7	12-20	applied in 1929		0.70	0.0504	8.78	
6	0-7	Lime and manure	7.50	1.70	0.1036	45.74	12
6	7-12			0.80	0.0532	10.08	
6	12-20			0.56	0.0392	8.24	
10	0-7	Check	6.72	1.28	0.0784	17.95	7
10	7-12			0.66	0.0392	8.03	
10	12-20			0.65	0.0364	8.37	
<i>Illinois Station—Morrow Plots</i>							
3 N.E.	0-8	Check, corn continuous	5.30	2.10	0.1260	31.69	11
3 N.E.	8-19			1.60	0.1170	16.96	
3 N.E.	19-30			0.90	0.0700	18.19	
3 S.E.	0-8	Manure, lime, phosphorus, corn continuous	5.50	2.40	0.1960	35.40	50
3 S.E.	8-19			1.80	0.1372	12.55	
3 S.E.	19-30			1.10	0.0700	16.27	
4 S.E.	0-8	Manure, lime, phosphorus, corn-oats	6.00	3.10	0.1848	27.18	55
4 S.E.	8-19			2.10	0.1568	11.08	
4 S.E.	19-30			2.00	0.1288	10.54	
<i>Missouri Station—Sanborn Field</i>							
17	A <sub>1</sub>	Corn continuous	5.35	0.97	0.0672	39.50	10
17	A <sub>2</sub>		4.35	0.90	0.0644	14.77	5
17	B			0.80	0.0616	20.41	
17	C			0.90	0.0448	15.69	
18	A <sub>1</sub>	Manure, 6 tons a year, corn continuous	5.38	1.85	0.1232	49.60	28
18	A <sub>2</sub>		5.47	1.20	0.0896	14.91	10
18	B			1.15	0.0700	18.57	
18	C			1.00	0.0420	18.29	
13	A <sub>1</sub>	Corn, oats, wheat, clover, and timothy 3 years	5.80	1.40	0.1064	10.6	7
13	A <sub>2</sub>		4.55	1.00	0.0644	36.4	trace
13	B			0.80	0.0560	28.96	
13	C			0.80	0.0392	18.15	

\* No longer used as a check, lime accidentally applied in 1929. Plats 6, 7, and 10 started 1907; rotation corn-oats-clover.

† Plats 14 and 15 started 1897; rotation corn-wheat-clover.

C. Burnham made pH determinations, and L. Wursten, other determinations.

out irrigation, and a small increase in base exchange capacity appears to have occurred with these soil building treatments at least under rainfall farming. Crop data were previously reported (7).

*Soil change in fertility plats at Astoria, Oregon.* Further evidence of the effect of different soil treatments in maintaining soil fertility is found in the analytical data from certain plats of the Astoria, Oregon Branch Experiment Station. The soil is "Red shot" land developed under annual rainfall of approximately 100 inches so that the untreated soil is distinctly acid. The mineralization of manure applied or the application of lime has tended to decrease the acidity of these plats. These treatments have apparently increased the phosphate supplying power and, by aiding the growth of clover grown in a rotation with grain and roots on this land, have generally increased the nitrogen and organic

TABLE 2

*Value of rotation, manure, and irrigation for maintaining fertility of soils*

Bean rotation, irrigation field, Oregon Agricultural Experiment Station, Corvallis, Oregon, maintained 20 years; surface 0-7 inches.

TREATMENT	TOTAL NITROGEN	ORGANIC MATTER	BASE EXCHANGE CONTENT
	<i>per cent</i>	<i>per cent</i>	<i>m.e.: 100 gm.</i>
Irrigated:			
Beans continuous.....	0 165	2.70	7 92
Rotated.....	0 189	2 60	8 15
Rotated and manured.....	0.221	3 30	8 32
Dry:			
Beans continuous.....	0 147	2.20	6.46
Rotated.....	0 174	2 50	7 47
Rotated and manured.....	0 195	2.60	7.78

matter content. Both lime and manure have resulted in an increased base exchange capacity and the calcium has markedly increased the exchange calcium of the soil. No correction for calcium carbonate on base exchange content was made. Sulfur has not improved this acid soil and has apparently decreased its base exchange capacity (table 3).

*Soil changes in fertility plats at Union, Oregon.* In the second section of table 3 will be found chemical characteristics of soil samples representing fertility plats of the Union Branch Experiment Station. The soil is Catherine silt loam developed under arid conditions and is of the nature of meadow land, being affected by ground water and having a good native supply of organic matter. The soil is nearly neutral in reaction but tends to become slightly acid with continued use of sulfur. The manure and complete fertilizer on this soil have resulted in an increase in total nitrogen and in organic matter. The legumes respond well to the sulfur treatment on this soil with a resulting increase in nitrogen and organic matter. There has been a slight build-up

in base exchange content where manure or complete fertilizer has been regularly applied. With the sulfur treatment no increase, even possibly a slight decrease, in base exchange capacity is indicated.

*Soil changes in fertility plats at Burns, Oregon.* The third section of table 3 shows characteristics of plat soils from the Harney Branch Experiment Station

TABLE 3  
*Value of various treatments for maintaining soil fertility*

PLATS	TREATMENT	pH	AVAILABLE PHOSPHORUS	TOTAL N	ORGANIC MATTER	BASE EX- CHANGE CON- TENT	EXCHANGEABLE		
							Ca	Mg	K
			lbs ac.	per cent	per cent	me · 100 gm			
<i>Astoria, Oregon Branch Experiment Station fertility plats*</i>									
1, 11, 21	Untreated	4.80	43	0.329	7.19	8.35	4.92	2.90	0.53
2, 12, 22	Manured	4.98	65	0.352	8.69	9.47	5.92	3.05	0.50
3	Limed	5.91	40	0.393	8.89	17.75	14.80	2.75	0.20
4	Manure and lime	5.72	70	0.378	6.96	14.00	10.65	2.95	0.40
6	Manure, lime, and plats	5.73	75	0.378	6.74	13.60	9.80	3.20	0.60
19	Sulfur	5.05	40	0.292	8.03	6.93	4.00	2.60	0.33
<i>Union, Oregon Branch Experiment Station fertility plats†</i>									
1, 5, 9	Untreated	6.8	†Doubtful	0.216	4.61	44.06	34.10	9.13	0.83
12	Manured	7.1	Medium	0.221	4.91	56.23	38.14	14.61	3.48
11	N, P, and K	7.1	Medium	0.224	4.58	50.51	34.86	14.70	0.95
7	Sulfur	6.5	Doubtful	0.229	4.71	43.97	33.00	9.66	1.31
2	Phosphorus	7.0	Medium	0.201	4.26	45.24	36.80	6.97	1.47
<i>Harney Branch Experiment Station, Burns, Oregon‡</i>									
2, 6	Untreated	7.58	116	0.239	5.04	32.19	19.70		
7	Manured	7.72	136	0.291	5.50	46.08	21.50		
10	N, P, and K	7.42	120	0.258	4.68	35.99	21.00		
4	Sulfur	7.35	112	0.241	5.18	36.21	21.00		

\* Surface soils (0-7 inches), humid section—maintained 12 years.

† Semi-arid section—maintained 13 years (Ill. Test).

‡ Arid section—maintained 13 years.

located at Burns, Oregon, under an arid climate with about 8 inches annual precipitation. The soil is dark very fine sandy loam and faintly alkaline. Manure and complete fertilizer appear to have increased the available phosphate and total nitrogen content. The manure has increased the organic matter content. Both treatments appear to have increased the base exchange capacity (the manure very definitely so) and also the exchangeable calcium. Sulfur is moderately beneficial to legumes on this soil, although the irrigation

water, which contains some sulfates, also improves the reaction of this faintly alkaline soil so that a larger accumulation of organic matter and nitrogen may form.

*Soil changes in plats under dry-farming at Moro, Oregon.* Effects of various soil treatments on soils from the dry land branch experiment station at Moro, Oregon, are presented in table 4. The annual precipitation at this station is about 12 inches, and humification of grain straw is slow. It has been possible to raise field peas annually with a tendency toward increased yields. Alfalfa decays rather slowly under the conditions. It appears from the data in table 4 that humus building under such drouthy conditions is a slow process. The only definite progress indicated by the data results from the use over a 21-year period of green peas each 4 years in comparison with the standard alternation of wheat with fallow. Green peas appear to have increased organic matter,

TABLE 4

*Effect of various treatments on soils from the branch experiment station, Moro, Oregon*

Sampled June, 1932, by Powers and Oveson. Determinations from surface samples, 0-8 inches.

PLAT	TREATMENT	ORGANIC MATTER	TOTAL NITROGEN	BASE EXCHANGE CONTENT
		<i>per cent</i>	<i>per cent</i>	<i>m.e. per gm.</i>
1068	Check—Crop residue experiment	2.10	0.0868	0.1174
1067	Strawy manure each 2 years	1.80	0.0980	0.1316
1069	Straw returned, 9 years	1.80	0.0952	0.1318
783	Wheat-Fallow Check	1.60	0.0728	0.1140
785	Green peas each 4 years, 21 years	1.90	0.0756	0.1408
Check; wheat-fallow by alfalfa land		1.70	0.0784	0.1250
Alfalfa ground—New grain; 5 years alfalfa, plowed 4 years; 2 crops, 2 fallows		1.70	0.0756	0.1252

nitrogen, and base exchange capacity. This is in agreement with controlled plant house and laboratory studies (3).

*Soil changes due to reclamation of alkali land, Vale, Oregon.* Soil changes due to reclamation of black alkali land are indicated by the data from a few selected plats as shown in table 5. Sulfur used in combination with manure has been effective in reclaiming virgin black alkali land when provided with deep drainage and copious irrigation. A nearly perfect stand of alfalfa has been established, and normal yields are now obtained. The check plats given similar treatment in every way except for omission of fertilizer or chemicals are still almost devoid of vegetation. It will be noted that the organic matter content of the treated plats has been practically doubled and that this increase is traceable to a depth of 20 inches in some cases. The nitrogen content has been increased from 50 to 100 per cent in the surface soil and has been definitely increased to the 20-inch level. Substantial increase in base exchange capacity is also indicated. The data reported elsewhere show that the char-

acter of the exchange bases has changed so that some 80 or 85 per cent of the exchange sodium has been replaced by exchange calcium, and the soil restored from a hard sodium clay to a friable fertile calcium clay.

TABLE 5

*Soil changes due to reclamation of black alkali land, Vale, Oregon experiment field*  
Sampled fall, 1932—Maintained 11 years

PLAT	DEPTH	TREATMENT	ORGANIC MATTER	TOTAL NITROGEN	BASE EXCHANGE CONTENT
	<i>inches</i>		<i>per cent</i>	<i>per cent</i>	<i>m.e.: 100 gm.</i>
A	0-5	S, 1.5 tons and manure, 10 tons, first 6 years	2.35	0.0728	16.75
	5-20		1.00	0.0336	19.27
	20-40		0.80	0.0280	23.30
B	0-5	Check	1.33	0.0532	14.99
	5-20		1.00	0.0280	22.54
	20-40		1.00	0.0336	25.65
C	0-5	S, 0.75 tons and manure 8 tons, first 5 years	2.75	0.0616	17.70
	5-20		0.87	0.0308	20.42
	20-40		1.00	0.0280	23.24
E	0-5	Check	1.49	0.0392	5.31
	5-20		1.00	0.0224	16.19
	20-40		1.00	0.0308	24.88

## DISCUSSION

Formerly Gedroiz (2) held that if the base exchange capacity of a soil became greatly reduced it could scarcely be restored. Recent discoveries (6) of the great base exchange capacity of the organic fraction of soils and of the effect of lime or a slight alkalinity in increasing absorptive capacity of the soil, however, betoken good progress in the restoration, or methods of conservation, of a base supplying power of a soil. Recent studies indicate more clearly the stable nature of the black earths where the given supply of organic matter is stabilized by the calcareous nature of the soil as well as by the continental semi-arid climatic conditions.

The studies herein reported indicate that the constructive humus building soil treatments, the employment of which in long-time field experiments has been associated with maintenance of good crop yields (7), have also resulted in increases in soil nitrogen, organic matter, and base exchange content. Fertilizers, where helpful to growth, have assisted in this humus building process. Livestock farming may aid maintenance of organic matter, whereas cultivation or uncontrolled grazing may lead to physical deterioration or increased erosion. The tendency for soil to deteriorate is most marked in humid climates where liming and perhaps mineral fertilizers may be necessary for success with legumes

in crop rotation. Further indications are that soil building along these lines is more readily accomplished in calcareous arid or semi-arid irrigated soil of medium texture and in a fairly cool climate. In an intimate neutral mixture of clay and humic colloids surface condensation may occur. Possibly molecular rearrangements involving splitting of double valence bonds may take place or addition compounds form between the clay acidoid and the ligno-protein nuclei, thus stabilizing or increasing base exchange capacity (4).

Russell (8) recognizes four sets of conditions: (a) Losses exceed returns with podzolization, (b) losses about balance returns in the brownearths, (c) losses are small or negligible with semi-arid grass lands in the stable black earth region, and (d) rainfall is insufficient for much growth, little humus is formed, and salts remain. Increase from maintenance of active nitrogenous organic matter may be expected to favor nitrification and sulfofication processes and even increase the phosphate supplying power of the soil. Increasing anion concentration under conditions favorable for activity of beneficial microorganisms of the soil should in turn result in bringing and holding nutrient bases in the soil solution, especially if a good supply of readily ionizable or exchange bases is present. Irrigation water may either increase or decrease the supply of soil nutrients, depending upon the quantity used and the composition of the water. Where only sufficient water is added to saturate the soil without drainage, plant food remains. The extent of leaching has been reported by Scofield (9) at the Grand Canyon Gaging Station for 5 years ending 1930, where the annual discharge has been 15,700,000 acre feet, carrying 12,000,000 tons of dissolved salts. Scofield discusses the possible base exchange reactions occurring in soil due to irrigation, particularly the replacement of earthy bases by alkaline bases, which are contained in some irrigation water with the consequent impairment of the physical conditions of the soil. Soil deterioration is more likely to result in a period of depression. Fortunately the increase from a humus building crop rotation comes with little or no cash outlay and frequently results in a decreased unit cost of production.

#### SUMMARY

A study has been made of certain chemical characteristics of soil samples collected from some of the oldest experimental plats of Oregon and of the eastern states. The long-continued use of manure, crop rotation with legumes, or liming in the case of acid soils has been associated with a definite increase in total nitrogen, soil organic matter, and base exchange content. It appears that the build-up or conservation of the soil's nutrient supplying power is more readily accomplished in the calcareous irrigated soils under a fairly cool climate. The results of the study should be of value in improving soils, in conserving soil fertility, or in guarding against soil degeneration.

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# PHOSPHATE FIXATION AND PENETRATION IN SOILS<sup>1</sup>

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Experience has shown that many soils, particularly those of the lateritic group, although very low in readily available phosphorus, give little response in crop yields when soluble phosphate is applied. Because of these results, the conclusion has often been reached that these soils are not deficient in readily available phosphorus. Experimental work indicates that this lack of response may be due to a rapid fixation of the applied phosphorus into forms that are difficultly available to plants.

Ford (1, 2) studied the fixation and availability of applied phosphates, and found a tendency in certain soils toward a lack of response from phosphate fertilization, due to a change in the form of the applied phosphorus. His work, together with that of others, indicates that certain iron and aluminum minerals in the soil are largely responsible for this change. The reader is referred to his work for a review of the literature. A brief summary of the salient facts on the fixation of phosphorus in soils follows.

## THEORY OF PHOSPHATE FIXATION

Phosphate fixation in soils may be purely chemical, the phosphorus taking the form of slightly soluble mineral phosphates, or it may be of a biological nature in which the phosphorus exists, first as living and later as inanimate organic or micro-organic material. This latter form of fixation is involved in the biological balance (4), and is very important in its relation to phosphorus availability to plants, but will not be considered further at this time.

On the basis of previous work, it appears that phosphorus is fixed in the soil largely through reactions with active calcium, iron, or aluminum. In this relation, these elements may be regarded as active when they unite chemically with phosphorus. Active calcium may be present in the soil in the exchangeable form, as calcium carbonate, or as any soluble calcium salt. Active iron and aluminum are believed to exist in the soil largely in the forms of the hydrated oxides. Although aluminum may be found in the solutions of mature soils, iron is rarely present in this way, except in mere traces. It is not necessary, however, for these

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<sup>1</sup> Contribution from the department of soils, University of Wisconsin, Madison, Wisconsin. Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

After this paper was written, work was reported by V. L. Weiser (Fixation and penetration of phosphates in Vermont soils. *Vt. Agr. Exp. Sta. Bul.* 356), which confirms with soils from Vermont the findings reported in this paper. For an excellent review of the literature, the reader is referred to this work of Weiser.

<sup>2</sup> The writer is appreciative of the kindly cooperation of Prof. E. Truog in connection with this study.

elements to be in solution in order to react with soluble phosphates to form phosphates of low solubilities, since the natural hydrated oxides react directly, as shown by Ford (2).

Figure 1 shows graphically how varying proportions of active calcium, iron, and aluminum in soils may determine the amounts of the different phosphates formed through fixation, and hence the availability of the applied phosphate. This figure is based upon general information and not upon specific data, and is presented to illustrate the relation of these elements to the fixation of phosphorus in soils.

With sufficient calcium and relatively small amounts of active iron and aluminum in the soil, most of the phosphorus is fixed as tricalcium phosphate. This phosphate is only slightly soluble in water, but easily soluble in 0.002 *N* sulfuric acid buffered to pH 3.<sup>1</sup> In this form, the phosphorus may be regarded as readily available for plant growth. Soils that fix phosphorus in this form are usually basic or only very slightly acid, and apparently contain relatively low amounts of active iron or aluminum. Miami silt loam (fig. 1, B) is a soil showing this type of fixation.

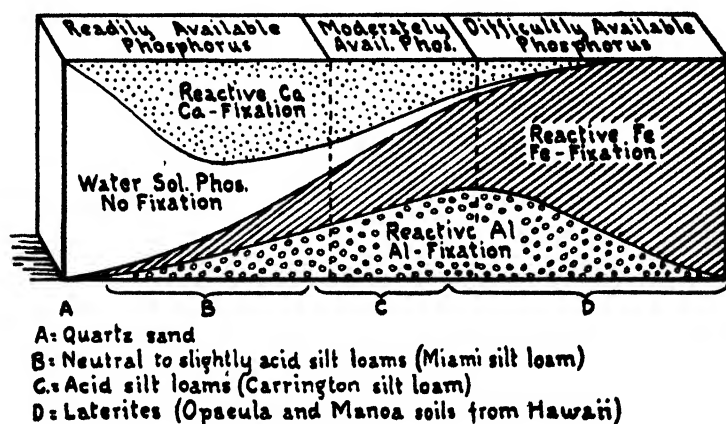


FIG. 1. THE RELATION OF CALCIUM AND ACTIVE ALUMINUM AND IRON TO THE FORM AND AVAILABILITY OF APPLIED PHOSPHORUS FIXED IN THE SOIL

When active calcium is low and the active agent of fixation consists largely of iron with some aluminum, the phosphorus is fixed, for the most part, as iron phosphates. The normal iron phosphate and the basic iron and aluminum phosphates are only very slightly soluble in 0.002 *N* sulfuric acid, and, for this reason, these three forms of fixed phosphorus may be regarded as difficultly available. Laterites (fig. 1, D) usually exhibit this type of fixation because of the high content of hydrated iron and aluminum oxides.

The normal aluminum phosphate is sufficiently soluble in 0.002 *N* sulfuric acid to justify its being classed as moderately available. Fixation in this form generally takes place in soils that are distinctly acid, because of the tendency of these soils to contain soluble aluminum, and also because the low pH value favors the combination of phosphorus with aluminum rather than with calcium. Carrington silt loam (fig. 1, C) tends toward this type of fixation.

In soils with little or no active calcium, iron, or aluminum, the soluble phosphate tends to remain soluble and be subject to leaching. This condition, however, is only encountered in soils made up largely of quartz (fig. 1, A).

<sup>1</sup> This 0.002 *N* sulfuric acid is buffered to pH 3 with potassium sulfate, and in this paper will be referred to as "0.002 *N* sulfuric acid."

It is thought that magnesium, manganese, and titanium may also function to some extent in the fixation of phosphorus, but their importance is questionable, except in very special cases.

Fixed phosphorus may then be regarded as falling into three groups; (a) the readily available  $[\text{Ca}_3(\text{PO}_4)_2]$ , (b) the moderately available  $[\text{AlPO}_4]$ , and (c) the difficultly available forms  $[\text{FePO}_4, \text{Al}_2(\text{OH})_2\text{PO}_4, \text{Fe}_2(\text{OH})_2\text{PO}_4]$ .

Considering these facts, it was the purpose of this work to determine more definitely the nature of the phosphates formed in soils through fixation of applied soluble phosphates.

#### EXPERIMENTAL

##### *Nature of the phosphates formed through fixation in the soil*

The nature of the phosphates formed by fixation in soils was studied by comparing the solubilities or the rate of solution curves of different phosphates with those of the phosphates in soils before and after treatment with soluble phosphate. The data for these curves were obtained by leaching small amounts of the different materials with 0.002 *N* sulfuric acid. The leaching was done at a uniform rate of 25 cc. an hour. The method of determining readily available phosphorus was essentially that of Truog (7). The data for the known phosphates and those for soils are shown graphically in figure 2.

*Solubility of known phosphates.* The curves in figure 2 were plotted from data taken at intervals of 2 hours, and represent the rate at which phosphorus was dissolved from the various materials during a 12-hour period. The area under the curve for any substance represents the amount of phosphorus dissolved from that substance in any given unit of time, and in this respect each graph is a two dimensional figure.

When tricalcium phosphate or finely ground rock phosphate was subjected to this leaching process, there was complete solution of the phosphorus in the first 50-cc. portion, or in 2 hours' time. If the amount of phosphorus going into solution in any one unit of the 2-hour period is proportional to the amount still undissolved, then the straight line of curve 1 represents the rate of solution of tricalcium phosphate or finely ground rock phosphate when leached in this way. The curves for tricalcium phosphate and for rock phosphate coincide, and in each case the total of 500 p.p.m. of applied phosphorus was recovered.

Aluminum phosphate dissolves much more slowly than calcium phosphate, and, with the exception of the first period, the curve for this substance (curve 2) is nearly a straight line, somewhat parallel with the base line, but slowly approaching it as leaching proceeds. The curve for ferric phosphate (curve 3) is much nearer the base line and practically parallel with it, whereas the curve for dufrenite  $[\text{Fe}_2(\text{OH})_2\text{PO}_4]$  (curve 4) practically coincides with the base line. With the exception of tricalcium phosphate and rock phosphate, each phosphate shows a distinctive solubility curve. With both the iron and aluminum phosphates, the first unit of time gave a greater solubility than the remainder of the curve would seem to indicate. This leads one to suspect either that perhaps both of these substances contained impurities as easily

soluble phosphate, or that hydrolysis took place much more rapidly during this period. The former view seems probably the more logical.

*Solubility of soil phosphates, and nature of fixed phosphorus.* Miami silt loam, a glacial soil from Wisconsin, and the Opauala soil, a laterite from Waialua, Hawaii, were each treated with 500 p.p.m. of phosphorus as mono-calcium phosphate, and allowed to stand for 2 days under optimum conditions of moisture and temperature. The soils were then dried and both the treated and untreated soils were leached with 0.002 *N* sulfuric acid. The results are shown in the second part of figure 2.

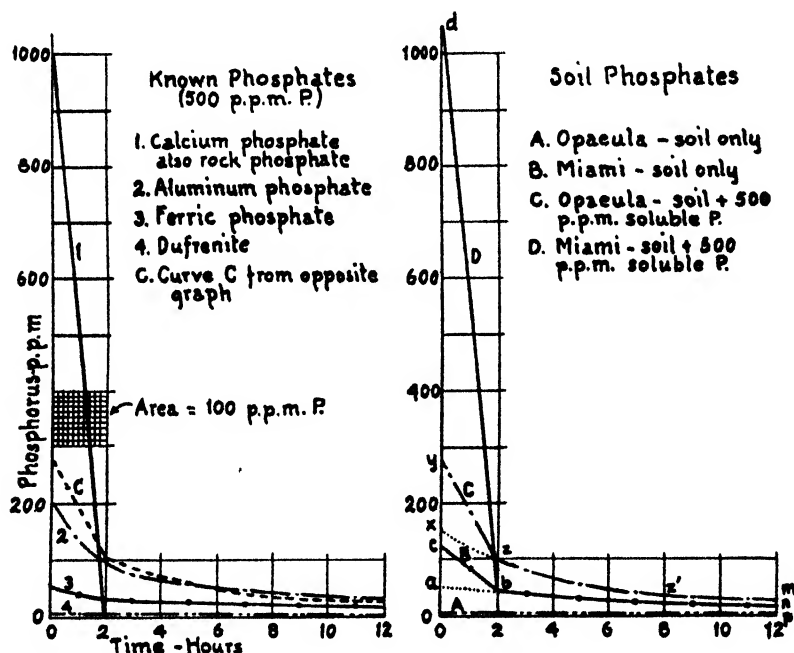


FIG. 2. RATE OF SOLUTION OR SOLUBILITY CURVES FOR KNOWN PHOSPHATES AND FOR THE NATIVE AND APPLIED PHOSPHATES IN SOILS (0.002 *N*  $H_2SO_4$  of pH 3)

Curve A for the untreated Opauala soil, very nearly coincides with the base line, which indicates that the form of the native phosphorus in this soil is similar to that in dufrenite. The form of the curve (B) for Miami silt loam, indicates that the phosphorus is present in this soil in at least three forms,—calcium, aluminum, and iron phosphates.

The recovery of phosphorus from the treated soils is shown in curves C and D. The treated Miami silt loam (curve D) gives a solubility curve during the first 2 hours similar to that for tricalcium phosphate or rock phosphate, and at point *b* this curve coincides with curve B and follows it to the end of the 12-hour period. This behavior may be interpreted to mean that the applied phosphate has been fixed in this soil largely as a calcium phosphate and is almost

entirely removed at the end of 2 hours' leaching. This fact, together with the coincidence of the two curves between  $b$  and  $n$ , shows that little or no iron and very little aluminum phosphate of any kind were formed. If the curve  $bn$  is projected to meet the ordinate, the triangle  $abc$  represents approximately the amount of readily available phosphorus in Miami silt loam before treatment, and the triangle  $abd$ , the amount after treatment with 500 p.p.m. of phosphorus as monocalcium phosphate. If there had been no fixation of the applied phosphorus in difficultly available form, the area of the triangle  $abd$  minus the area of the triangle  $abc$  should equal the 500 p.p.m. of phosphorus applied, and with the Miami silt loam used, this is true to the extent of about 95 per cent.

The results with the Opauala soil were entirely different, as is shown in curve C. First, there is much less phosphorus extracted during the first 2 hours than from the treated Miami silt loam, and, after this, there is no coincidence of curve C with the corresponding parts of curve A. The spread between  $op$  and  $zm$  indicates that a considerable quantity of the phosphorus applied to the Opauala soil has been changed to slightly soluble iron and aluminum phosphates. The arc  $zz'$  indicates aluminum phosphate, and the almost straight line  $z'm$ , iron phosphate. If the arc  $zz'$  is projected to meet the ordinate, the triangle  $xyz$  represents the readily available phosphorus which, in this case, is probably made up of both calcium and aluminum phosphates. The area  $yzmpo$  roughly represents the amount of the applied phosphorus recovered as calcium, aluminum, and normal iron phosphates, and the difference between this quantity and the 500 p.p.m. of applied phosphorus is that quantity which has gone over to the iron and basic aluminum phosphates.

If curve C is superimposed upon the left half of figure 2, its partial similarity to curves 1, 2, and 3 can be easily seen. This is an indication that it is a composite of these three curves, and that its form comes from varying amounts of these phosphates or phosphates of similar solubility.

Additional data obtained in a similar manner to that illustrated in figure 2 were obtained for the two soils already reported, and for Carrington silt loam, another glacial soil from Wisconsin, and the Manoa soil, another laterite from the upper Manoa valley, Hawaii. In this case the soils were treated with 400 p.p.m. of phosphorus in solution as monocalcium phosphate, boiled to dryness, and then leached in the way previously described. The amounts of the different fixed phosphates were then calculated from the solubility curves. Table 1 gives these data.

The phosphorus given in the last column of table 1 includes all of the calcium and a part of the more easily soluble aluminum and iron phosphates, and was obtained by a single 30-minute extraction with 0.002 *N* sulfuric acid, and a ratio of soil to solution of 1 to 400. Figure 3 shows graphically the relative amounts of the different phosphates formed by fixation in each of the four soils studied.

In the two glacial soils, a relatively large amount of the applied phosphorus is held in the calcium form. As the pH value of the soil becomes less, the

phosphorus fixed as calcium phosphate decreases, and the iron and aluminum or the difficultly available phosphates increase. In the Hawaiian laterites, the

TABLE 1

*Relative amounts of different phosphates formed and the amounts of readily available phosphorus recovered from soluble phosphates applied to soils*

SOIL	pH	APPLIED PHOSPHORUS			
		Changed to forms indicated			Recovered as readily available by 0.002 N $H_2SO_4$ extraction
		Calcium phosphate (Readily available)	Aluminum and iron phosphates (Moderately available)	Basic aluminum and iron phosphates (Difficultly available)	
		per cent	per cent	per cent	per cent
Miami silt loam.....	6.66	83.5	7.5	9.0	85.5
Carrington silt loam.....	5.67	52.0	26.7	21.3	68.7
Hawaiian, Opauala.....	6.09	2.0	27.0	71.0	19.5
Hawaiian, Manoa.....	5.40	0.0	Not determined	Not determined	8.7

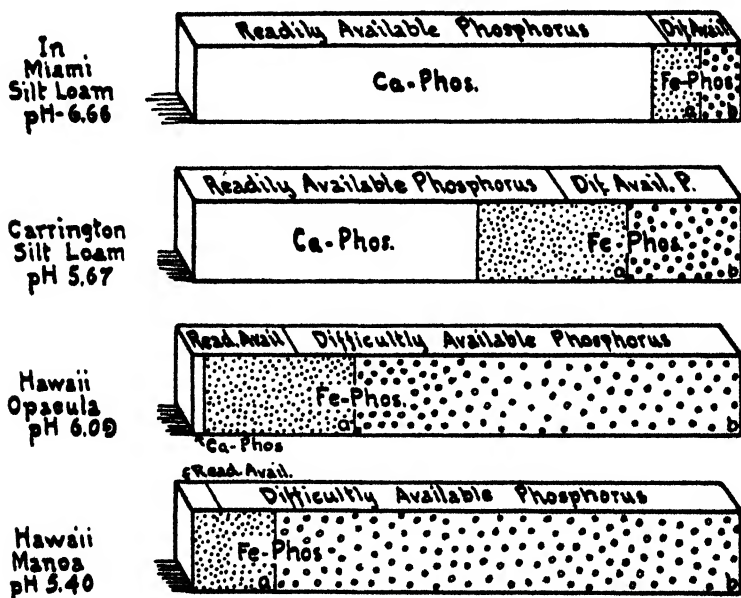


FIG. 3. THE RELATIVE AMOUNTS AND AVAILABILITY OF THE PHOSPHATES FORMED IN DIFFERENT SOILS FROM APPLIED SOLUBLE PHOSPHATE

phosphates of iron and aluminum or the difficultly available forms make up by far the greater part of the fixed phosphorus. Although the pH value of the soil is an important factor in determining the form in which the phosphorus is

fixed in a soil, this factor may be largely overshadowed by the presence of large amounts of hydrated oxides of iron or aluminum.

In figure 3, the part designated as iron phosphate includes not only the iron but also the aluminum phosphates. The *a* fraction may be spoken of as being moderately available, and is perhaps made up largely of aluminum phosphate together with the more soluble fraction of the iron phosphate. The possibility of phosphorus in organic combination making up a portion of this fraction is eliminated by the conditions under which the soluble phosphate was fixed in the soil. The *b* fraction is made up largely of the basic phosphates of iron with some basic aluminum phosphate and some normal iron phosphate. This division point shifts as the equilibrium changes, with the tendency for the more difficultly available phosphates to increase.

#### *Penetration of applied phosphates*

Aside from the form, the position in which the applied phosphorus is fixed in the soil is also important. In order to be available for plant growth, the phosphorus must be within the root zone. Soils vary greatly as to the extent to which they permit the penetration of soluble phosphates. The extent of penetration is governed largely by the nature and the amounts of the materials in the soil which are capable of precipitating the soluble phosphate.

The four soils previously described were used for a study of positional fixation. Tubes about 2 inches in diameter were filled with these soils to a depth of 5 inches. Enough phosphorus (42 mgm.) in solution as monocalcium phosphate to make up approximately 225 p.p.m., based on the entire soil mass, was placed on the surface of the soil, and 6 inches of irrigation water was applied. The phosphorus leaching through the soil and the amounts of readily available fixed phosphorus in the various strata of these soil columns were determined. In table 2 are given the amounts of the applied phosphorus recovered from the soil in readily available form and also the place where it was found after fixation. Figure 4 shows in graphic form the penetration of phosphorus into each of the four soils studied.

Figure 4 is constructed on a two dimensional basis, and the amounts of the different phosphates represented are proportional to the areas shown. Of the phosphorus fixed in the soil, the position and quantity of the readily available forms only are shown, and it is assumed that the difficultly available phosphorus is fixed approximately in the same position.

The greatest penetration of phosphorus in these four soils occurred in the Miami silt loam and the least in the two Hawaiian laterites, with the Carrington silt loam falling intermediate. The water-soluble phosphorus passing through 5 inches of soil also stands in this same relationship. The Miami silt loam apparently contains relatively little active iron or aluminum and not more than a trace of calcium carbonate. With these factors balanced in this way, this soil is almost ideal for the effective use of soluble phosphates. There is very little difficultly available phosphate formed, and there is sufficient ex-



TABLE 2

*Readily available phosphorus recovered at different depths and from drainage water, from soluble phosphate applied with irrigation to the surface of soils*

	READILY AVAILABLE PHOSPHORUS RECOVERED FROM 42 MGM. OF APPLIED PHOSPHORUS			
	Miami silt loam	Carrington silt loam	Hawaiian Opauala	Hawaiian Manoa
	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
Recovered from soil				
First half inch. . . . .	4 02	6 82	13 20	2 94
Second half inch. . . . .	2 42	2 62	1 07	0 00
Total first inch. . . . .	6 44	9 44	14 27	2 94
Second inch. . . . .	4 84	5 12	0 00	0 00
Third inch. . . . .	4 84	4 61	0 00	0 00
Fourth inch. . . . .	4 84	3 54	0 00	0 00
Fifth inch. . . . .	4 84	2 94	0 00	0 00
Total recovered from soil. . . . .	25 80	25 69	14 27	2 94
Recovered in the drainage water . . . . .	15 50	2 30	0 00	0 00
Total recovered in readily available form . . . . .	<i>per cent</i> 98 4	<i>per cent</i> 66 6	<i>per cent</i> 34 0	<i>per cent</i> 7 0

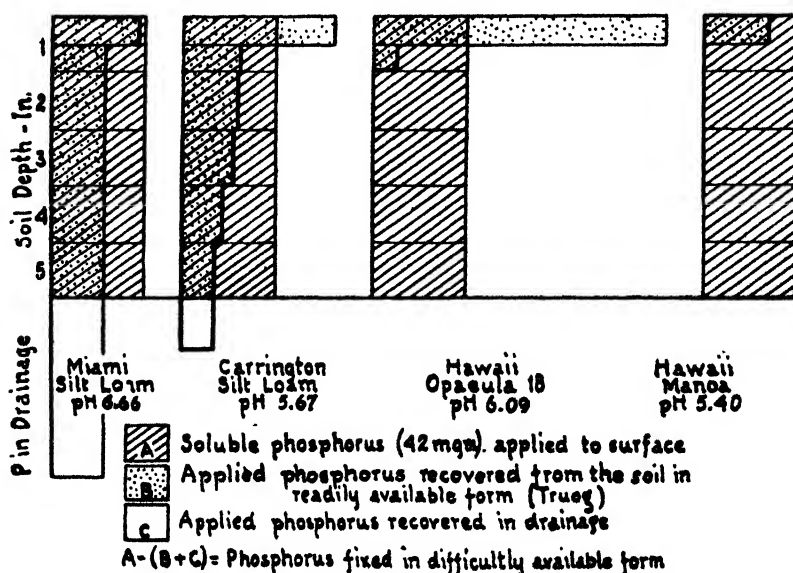


FIG. 4. THE DOWNWARD PENETRATION OF PHOSPHORUS AND ITS RECOVERY IN READILY AVAILABLE FORM (TRUOG) WHEN SOLUBLE PHOSPHORUS IS APPLIED WITH IRRIGATION TO THE SURFACE OF 5-INCH DEPTHS OF DIFFERENT SOILS

changeable calcium present to prevent excess leaching, and, at the same time, not enough calcium carbonate to hold the phosphorus in the immediate surface soil. As a result, a good positional fixation is obtained.

As soils become more acid and the pH value drops below 6.5, there is a greater tendency for the iron and aluminum to unite with the phosphorus. This is due, in part, to a tendency for a greater amount of active iron and especially aluminum to be present in the more acid soils, and, in part, to a smaller amount of calcium in the fixation equilibrium, thus giving the iron and aluminum a better chance to react with the phosphorus. This appears to be true of the Carrington silt loam which has a pH value of 5.6. As compared with the Miami silt loam, there is a very much greater fixation of phosphorus in the difficultly available form, and, at the same time, a very much greater tendency to hold the readily available phosphorus in the surface soil.

If the soil should contain a considerable amount of calcium carbonate, the phosphorus would be held largely in the immediate surface soil as tri-calcium phosphate. In this case the form of the fixed phosphorus may be satisfactory from a purely chemical standpoint, but the excess of lime present and the nearness of the fixed phosphorus to the surface of the soil render this type of fixation less desirable.

The laterites considered in figure 4 allow only a very slight penetration of the soluble phosphate (even though the physical condition is ideal for its movement). In neither laterite has the soluble phosphate penetrated more than half an inch. At the same time, there is a fixation in the difficultly available form of from 66 to 93 per cent of the applied phosphorus. As a rule, these soils contain a very low amount of dializable iron or aluminum, but apparently large quantities of hydrated oxides of these elements which react with soluble phosphate and not only hold it in the least soluble form, but also very near the surface. Compared with the glacial soils shown, the two laterites from Hawaii may truly be called high fixing soils, not only with respect to the form, but also to the position in which the phosphorus is fixed.

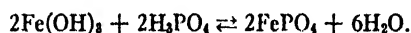
Stephenson and Chapman (6) found a varying penetration in the field, depending upon the kind of soil, the amount of the applied phosphorus, and the manner of its application. However, as a rule, it is generally thought that under actual field conditions the same soil will allow much less penetration than under ideal laboratory conditions, especially where the application in the laboratory is made in solution and followed immediately with large amounts of irrigation water.

#### DISCUSSION

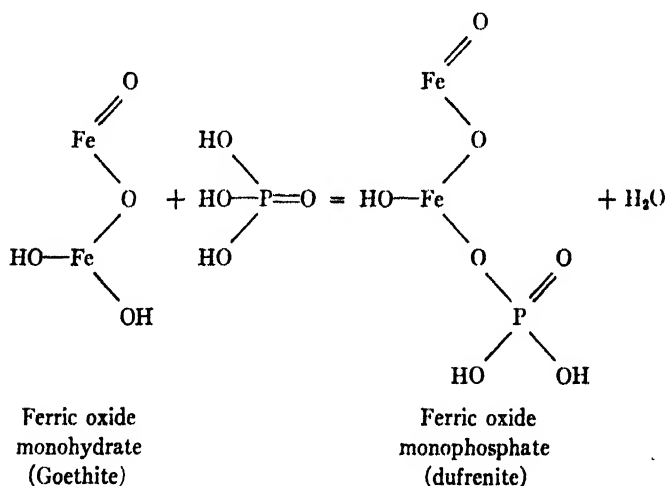
The work of Gaarder (3) indicates that the pH value of the soil is an important factor influencing the forms in which phosphates are fixed in soils. He reports that the amount of phosphorus going into solution from calcium phosphate at pH 6.5 is only about one-third that from normal aluminum phosphate and only about one-fourth that from normal iron phosphate, whereas at pH 5.5

the phosphorus from calcium phosphate has from 3 to 5 times the solubility of that in the iron and aluminum forms. Based upon these facts, it appears that a lower pH value in a soil not only increases the solubility of the phosphorus from calcium phosphate but at the same time reduces the amount of calcium in the mass action equilibrium with phosphorus, both of which tend to increase the amount of phosphorus fixed in difficultly available form.

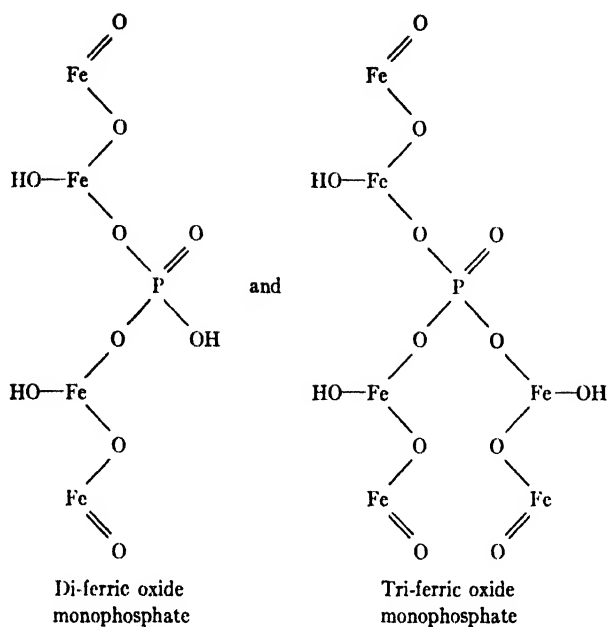
The fixation of phosphorus as iron or aluminum phosphates does not necessarily depend upon iron or aluminum in the soil solution, but rather upon the state of hydration of their oxides. If the iron oxide is fully hydrated, the normal phosphate may be formed:



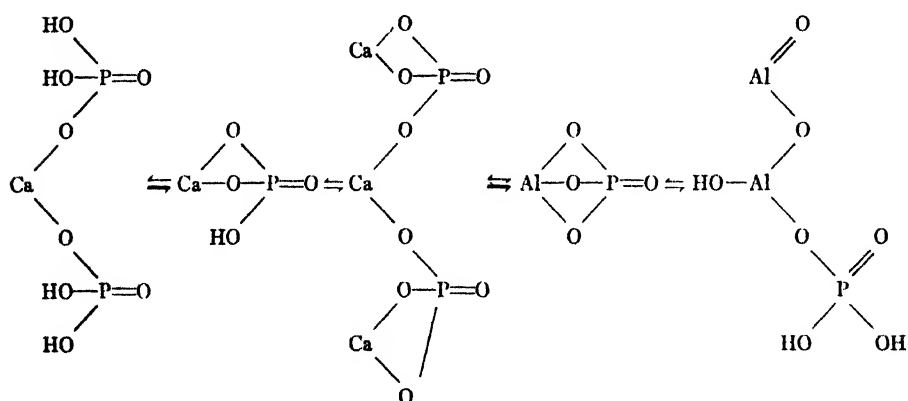
There is some question, however, as to the extent of this reaction, because of the instability of the tri-hydrated iron oxide and its tendency to change to the monohydrate, which is the more stable form. Ford (2) has shown that dehydrated iron oxide (hematite,  $\text{Fe}_2\text{O}_3$ ) does not react with soluble phosphates, but that ferric oxide monohydrate (Goethite,  $\text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}$ ) is very reactive. The reaction seems to be one of simple addition, making it unnecessary for the iron to go into solution, and may be illustrated as follows:

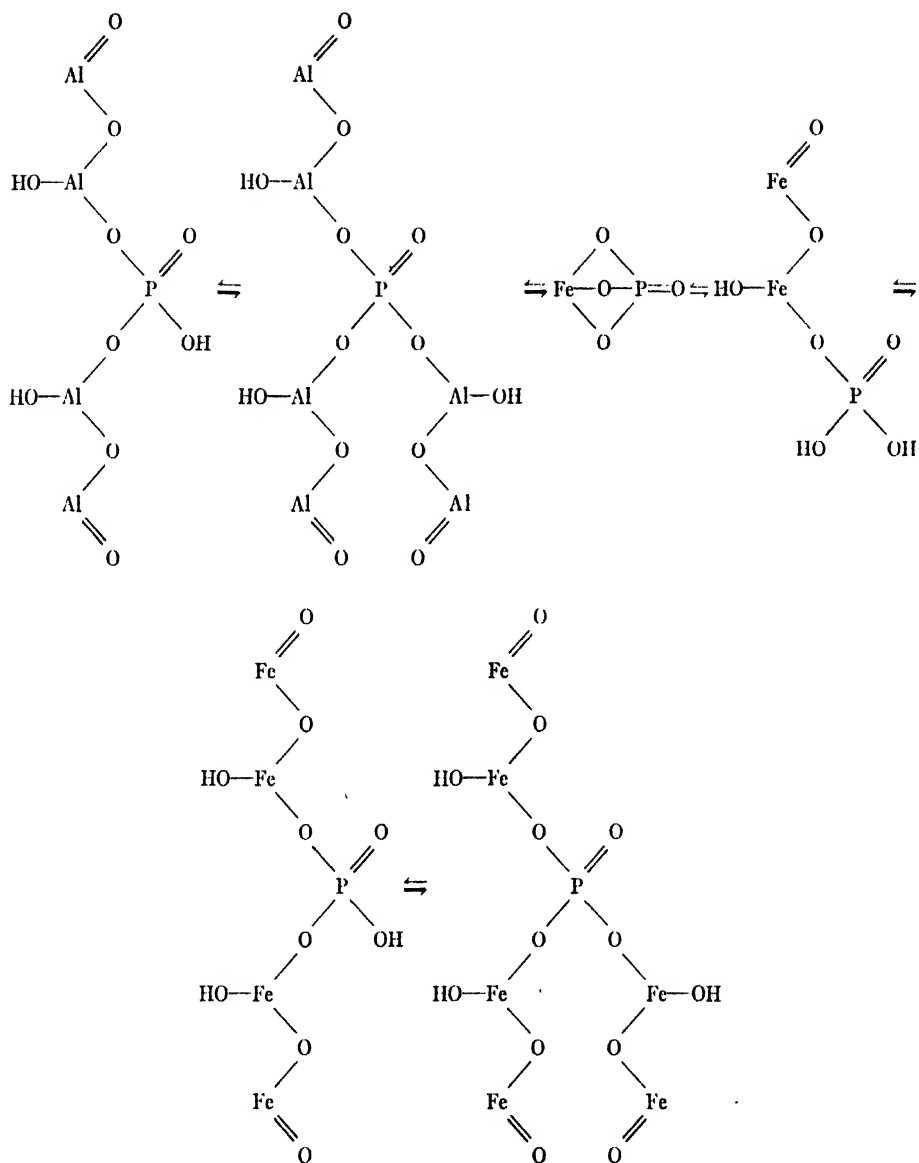


The work of Leahey (5) presents some evidence that there may be combinations of iron with phosphorus in which the ratio of ferric oxide to phosphoric acid ( $\text{P}_2\text{O}_5$ ) is four to one or even six to one. This would suggest at once the di- and tri-ferric oxide combinations with phosphoric acid, which may be represented as follows:



In view of the facts presented, it seems logical to think of phosphate fixation in soils as a change from the soluble to the relatively insoluble forms, and the establishment of a slowly changing equilibrium to which there is a tendency for the more soluble or more readily available forms to change over to the less soluble and more difficultly available forms. Somewhat in order of decreasing availability, such an equilibrium may be expressed as follows:





Such an equilibrium is highly theoretical, and is shown only to give an idea of some of the possible ways in which phosphorus may be combined in the soil in the fixation process. It will also serve to show how varying amounts of active calcium or of the hydrated oxide of iron or aluminum in the soil may influence the extent and kind of fixation taking place.

## SUMMARY

In this study of phosphate fixation, four rather different soils were used in an attempt to ascertain more definitely the nature of the compounds formed and the depth of penetration when a soluble phosphate was applied to these soils. The nature of the fixed phosphates was determined by means of comparative solubilities (in 0.002 *N* sulfuric acid) of known phosphates and those formed in soils through fixation. The results may be summarized as follows:

The predominating form in which soluble phosphate is fixed in the soil depends much upon the relative abundance in the soil of the different materials capable of fixing phosphorus. If the ratio of active calcium to active iron and aluminum is high, the fixation will be largely in the calcium form, and the fixed phosphorus will be readily available. If the reverse is true, the fixation will be largely as the iron and aluminum compounds of phosphorus, which are difficultly available.

On the basis of solubility, the soluble phosphate was fixed in the Miami silt loam largely as calcium phosphate. In the Carrington silt loam, which was more acid, less calcium phosphate and more iron and aluminum phosphates were formed. In the two Hawaiian laterites, the fixation was largely as the basic forms of iron and aluminum phosphates. Although the reaction and the presence of reactive calcium are important factors governing the forms of the fixed phosphorus, in the laterites these factors were overshadowed by the presence of very large quantities of the hydrated oxides of iron and aluminum.

The greatest phosphate penetration occurs in soils of neutral to slightly acid reaction which contain little active iron or aluminum. Either active calcium, iron, or aluminum inhibits the downward movement of phosphorus in soils in proportion to the amounts present and inversely proportional to the solubilities of their compounds with phosphorus. Of the soils studied, the slightly acid Miami silt loam with little active iron or aluminum gave maximum penetration, whereas the laterites, with large amounts of active iron and aluminum, allowed very little penetration, the phosphorus being fixed and held largely in difficultly available forms within a few millimeters of the point of application.

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# THE INFLUENCE OF THE HYDROGEN-ION CONCENTRATION OF THE CULTURE SOLUTION UPON THE ABSORPTION AND ASSIMILATION OF NITRATE AND AMMONIUM NITROGEN BY PEACH TREES GROWN IN SAND CULTURES<sup>1</sup>

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A comparison of various forms of nitrogen for the purpose of determining which form is the most satisfactory for plants in general, or for specific plants, has attracted considerable interest in the past. Recent investigations, however, have complicated considerably the problem of comparing different forms of nitrogen used for plant nutrition.

Thus the work of Stahl and Shive (23, 24) has shown that some plants may, during the early stages of their life cycle, absorb nearly all of their nitrogen in the ammonium form from nutrient solutions containing both ammonium and nitrate ions. However, as the plants increase in age, under the same set of experimental conditions, it was found that the rate of absorption of ammonium nitrogen decreases. The relative proportions of ammonium and nitrate nitrogen absorbed during various stages of the life cycle were found to vary considerably among different species. Pirschle (13), Tiedjens and Robbins (29), and Tiedjens and Blake (28), on the other hand, have shown recently that the ability of plants to grow in water or sand cultures containing either ammonium or nitrate nitrogen depends primarily upon the reaction of the nutrient solution used.

In the investigations reported herein, attempts have been made to study the influence of the hydrogen-ion concentration of the nutrient solution employed upon the absorption and assimilation of ammonium and nitrate nitrogen. Since herbaceous types of plants have usually been employed for absorption studies, it was considered desirable to use trees in these experiments. Preliminary work has shown that peach trees are well adapted for use in sand cultures, and that they grow rapidly and respond quickly to nutrient treatments. Accordingly, in the experiments described in the following pages, peach trees (*Prunus persica* Stokes) have been grown and studied in two series of treatments, one containing nitrogen only in the ammonium form and the other containing nitrogen only in the nitrate form.

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## EXPERIMENTAL METHODS

*Plant material*

Seedling peach trees were used in these studies in order to avoid variations in growth that may result from differences in the amounts of reserve foods present in older trees. It was recognized, of course, that the use of seedling trees might involve inherent variations in habit and vigor of growth, as well as possible differences in composition. As a result of peach breeding investigations conducted at the New Jersey Experiment Station for many years, the growth habits of seedlings from numerous varieties of peaches are well known. Accordingly, at the suggestion of Prof. M. A. Blake, seedlings grown from pits from an open pollinated Early Crawford tree were chosen. Seedlings from this variety are homozygous in many of their characters and are nearly uniform in their habits of growth. The method used for propagating the plants and handling them prior to the selection of a group of individuals for these experiments has been described in an earlier publication (1) and need not be repeated here.

For use in these studies, groups of uniform seedlings with tops about 15 cm. high were selected. They were transplanted into 2-gallon glazed porcelain percolators filled with carefully washed white quartz sand. These percolators were 24 cm. in diameter, 30 cm. in height, having perpendicular sides and a rounded bottom with a 2.5 cm. drain hole in the center, thus providing perfect drainage. Two plants were placed in each pot. The drain hole in the pot was covered with a watch glass which, in turn, was covered by a pad of glass wool. This arrangement prevented even the finest sand particles from washing through the hole, and at the same time it permitted free drainage.

*Culture solutions*

For the purpose of these studies, it was necessary to use two different series of nutrient solutions: one having all of its nitrogen in the ammonium form, and the other having all of its nitrogen in the nitrate form. Moreover, in order to permit an approximate comparison of the absorption and assimilation of these two forms of nitrogen, it was considered desirable to use culture solutions all of which contained, in so far as possible, the same concentrations of essential elements, especially nitrogen. The importance of such a balance is emphasized by the work of Prianischnikow (16, 17, 18), and of Holley, Pickett, and Dulin (5). It is not possible, however, to prepare two solutions having identical concentrations of nitrogen, one containing the nitrate ion and the other the ammonium ion, and still have identical concentrations of all other ions. Accordingly, the amounts of magnesium and sulfate ions were varied slightly, since it has been shown (1) that peach trees grow well over a very wide range in concentrations of these two ions. The similarity with respect to the ionic composition of the two series of solutions was changed further by the amounts of normal potassium hydroxide and hydrochloric acid added to obtain the desired hydrogen-ion concentrations.

Peach trees have been found (1) to grow especially well in sand cultures when supplied continuously with culture solutions containing medium to high concentrations of potassium and phosphate ions. A high concentration of phosphate in a solution having a low hydrogen-ion concentration, however, brings about an undesirable precipitation of calcium and magnesium. Therefore Number 12, or the  $T_3R_1C_3$  solution, of the ammonium sulfate series of Jones and Shive (7) was modified as shown in table 1 to meet the requirements of this investigation. Each of the modified solutions has an osmotic concentration of approximately 0.9 atmosphere.

Boron and manganese were supplied to all cultures by the addition of a solution of boric acid and manganese sulfate directly to the culture solutions at the rate of 0.5 p.p.m. of each of these elements. Normal HCl and KOH were used to adjust the solutions to the desired pH value. These culture solutions were supplied to the plants continuously by the method of Shive and Stahl (22).

TABLE 1  
*Composition of nutrient solutions*

	PARTIAL VOLUME MOLLECULAR CONCENTRATION	
	Ammonium series	Nitrate series
$KH_2PO_4$ . . . . .	.	0 00633
$K_2SO_4$ . . . . .	0 00316	. . .
$CaH_4(PO_4)_2$ . . . . .	0 00317	
$Ca(NO_3)_2$ . . . . .		0 00318
$Mg(NO_3)_2$ . . . . .	. . . . .	0 00120
$MgSO_4$ . . . . .	0 00711	0 00711
$NH_4OH$ . . . . .	0 00876	.

#### *Absorption tests*

The sand culture method of growing plants does not lend itself so readily to determination of the rates of ion absorption by plants as does the solution culture method; nor is it possible to determine these rates for sand culture plants with the same degree of accuracy as it is for plants grown in solution culture. This is due, of course, to the fact that, first, the plants grown in a sand culture cannot be removed from the sand and transferred to a separate and special culture vessel for the purpose of making a test; and, second, the culture solution used for the test cannot be removed quantitatively for chemical analysis from the sand in which the plants are rooted. Thus, an approach to accuracy in making absorption tests with plants grown in sand cultures appears to depend largely upon the use of a method which permits precisely similar treatment of every culture.

The method which was finally adopted for use in these experiments is described as follows: A rubber stopper containing a short length of glass tubing was inserted tightly into the drain hole of each culture vessel used for absorp-

tion tests. By means of a short length of rubber tubing, a culture was connected to a 1-liter suction flask, and most of the free nutrient solution held in the sand was withdrawn by suction. Following this, the sand was washed with a liter of distilled water added in four applications. The pot was allowed to drain for 15 minutes, and then suction was applied until only an occasional drop of liquid was withdrawn.

Exactly 1 liter of the respective test solution was supplied to the culture by the method of continuous solution renewal mentioned previously. All solution that leached through the culture during the test period was collected in a carefully cleaned granite-ware pan placed beneath the drain. The rate at which the solution was supplied during the test always corresponded to the rate at which the solution was supplied per day to that particular culture. Thus, if the culture had been receiving 4 liters of solution daily, the absorption interval was 6 hours.

Immediately after the termination of the absorption interval, the free nutrient solution held in the sand was withdrawn by suction, just as the solution was withdrawn preparatory to the test. This solution was added to the leachings. The sand was then washed with 1 liter of distilled water as before and allowed to drain for 15 minutes. The excess wash water was then withdrawn by suction as before and added to the leachings. The pan, suction flask, and rubber tubing were washed, and the wash water, together with the leachings, was made to comprise a volume of exactly 2 liters. The amount of moisture transpired by the plants during the test was usually far greater than the amount of water required to wash the apparatus.

By a comparison of the amount of nitrogen found in an aliquot of 2 liters of solution that passed through the cultures with that found in an equivalent aliquot of the initial solution, the approximate amount absorbed by the plants during the test was determined.

The treatments given the cultures before and after the test solution was applied were made as nearly alike as possible. Special care was taken to handle in precisely the same manner all cultures used in a given test. Of course, it is to be assumed that, at best, the method can give only an approximate measure of the nitrogen absorbed by the plants from the test solutions passing in a given interval of time through the sand in which the plants were rooted. Nevertheless, these approximate absorption rates of the various cultures should be comparable one with another.

In order to study the absorption of nitrogen by cotton seedlings grown in sand cultures, Naftel (9) used a method somewhat similar to the one described. He used only a kilogram of sand per culture, however, and after washing it with 500 cc. of distilled water to remove the nutrient solution, allowed it to drain overnight. By such treatment, he undoubtedly washed the free nutrient solution from the sand very thoroughly and obtained a satisfactory indication of the amounts of nitrogen absorbed by plants in the different treatments. Nevertheless, he subjected his plants to drastic changes in osmotic pressure

over comparatively long periods of time. It is probable that the method employed for these experiments with peaches avoided, or at least minimized, any injurious effects which this might have had upon the plants, and also that it minimized any tendency toward the excretion of ions from the roots.

#### EXPERIMENTAL RESULTS

Two sets of experiments, extending over a period of 2 successive years, were carried out. These will be referred to as the "1931 experiments" and the "1932 experiments," and will be treated separately. The 1931 experiments must be regarded in the nature of a preliminary investigation only, but since they brought out some important points, the data obtained will be presented and discussed in some detail. For the sake of brevity in considering results and the data to be presented, reference will be made to the various pH treatments and to the plants grown under them by the use of simple abbreviated expressions. Thus the pH-4 treatments in the ammonium series will be referred to as the " $\text{NH}_4$ -pH-4 treatments," and the plants grown under these treatments will be designated the " $\text{NH}_4$ -pH-4 plants." The corresponding treatments in the nitrate series will be called the " $\text{NO}_3$ -pH-4 treatments" and the plants will be designated the " $\text{NO}_3$ -pH-4 plants." Other treatments and the plants grown under them will be designated by corresponding terms relating to the pH of the culture solution and the series in question. In all cases sufficient plant material was produced under each treatment, both in the ammonium and in the nitrate series, to furnish adequate samples for chemical analyses for the purpose of determining the distribution of the nitrogen fractions in the plants.

#### *1931 experiments*

On June 7, a sufficient number of uniform seedlings with tops about 15 cm. high were selected and transplanted to the 2-gallon culture vessels previously described. The plants in the ammonium series were supplied by the continuous renewal method (22) with a solution containing the  $\text{NH}_4$  ion as the only source of nitrogen, and those in the nitrate series were supplied with a solution containing the  $\text{NO}_3$  ion as the sole source of nitrogen. Each of these solutions was previously known to produce excellent growth of peach trees, and it was supplied within a favorable pH range for normal development, so that the young trees in all the cultures of both series were very vigorous and in excellent condition when the final treatments were established on July 17. On this date the 21 cultures of each series were divided into three groups of 7 cultures each.

During the remainder of the growth period the cultures of the three groups in the ammonium series were supplied with the respective solution used in this series, adjusted to pH 4.0 for the first group, pH 6.0 for the second, and pH 8.0 for the third. The cultures of the three groups of the nitrate series were similarly treated with the respective solution employed in that series, adjusted to corresponding pH values. Each culture was continuously supplied with its

solution at the rate of 1 liter per 24 hours until August 5, when the rate of supply was increased to 2 liters per 24 hours, in order to obviate excessive reaction change of the culture solution as it passed through the sand. On August 15, the rate of supply was raised to 4 liters per culture per 24 hours.

*Growth responses and nitrogen absorption.* The growth responses of the peach trees in the various treatments were measured by three quantitative

TABLE 2

*Total linear growth in centimeters and green and dry weight yields in grams produced per plant in 1931 experiments*

DATE	INITIAL pH OF CULTURE SOLUTION	TOTAL LINEAR GROWTH OF STEMS	GREEN WEIGHT			Dry weight				
			Stems	Leaves	Stems plus leaves	Stems	Leaves	Roots	Whole plants	
Ammonium series										
August 1.....	{	4	187	7.55	16 28	23 83	2 48	3 68	1 38	7 84
		6	257	9 98	22 57	32 55	2 95	5.23	1 47	9 65
		8	197	7 72	17 07	24 79	2 15	8 19	1.58	7 92
August 14.....	{	4	205	6 55	16 40	22.95	2 48	4.68	2 15	9 31
		6	326	14 40	34 55	49 45	5 38	8.75	3 20	17 33
		8	271	12 28	27 40	39 68	4 08	6 35	2 45	13 08
August 25.....	{	6	491	23 80	46 35	70 15	8 35	11 73	6 75	26 83
		8	391	17 50	36 30	53 80	5 90	8 85	5 50	20 25
Nitrate series										
August 1.....	{	4	255	12 82	25.08	37 90	3 77	5 65	2 02	11 44
		6	280	12 92	24 02	36 94	3 87	5 42	2 13	11 42
		8	233	11 07	22 92	33 99	3 43	5.33	1.92	10 68
August 14.....	{	4	446	25.78	47 00	72 78	7 95	10 88	4 75	23 58
		6	415	23 73	44 15	67 88	7 83	10 30	5 20	22 83
		8	375	14 73	39 43	59 16	5 93	8 98	4 28	14.19
August 25.....	{	4	691	52 08	81.03	133 10	16.23	19 33	8 50	44 06
		6	497	35 98	64 60	100 58	11.50	15.23	8 13	34 86
		8	414	30 40	55.98	86.38	9 98	13 80	7.75	31 53

criteria. The first of these was the measurement made every 5 or 6 days, of the height of the main stem of each plant. These data are supplemented by measurements of the total linear growth of main stems and branches per plant, recorded at each harvest period. The second criterion was that of average green weight per plant per treatment recorded at each harvest period. The third, and perhaps the most important quantitative criterion, was that of average dry weight per plant per treatment determined at each harvest period.

The results of these measurements are presented in table 2, and those of the nitrogen absorption tests are given in table 3, in milligrams of nitrogen absorbed per gram of dry plant material per hour. These data will be considered only very briefly, since the 1931 experiments were preliminary in nature, and since the results agree in general with those obtained in the 1932 experiments, which will be considered in greater detail.

From the data of table 2, it will be observed that the plants in the ammonium series, judged by each of the three criteria, made the best growth in the  $\text{NH}_4$ -pH-6 treatment, the poorest growth in the  $\text{NH}_4$ -pH-4 treatment, and growth

TABLE 3

*Rates of absorption of ammonium nitrogen and of nitrate nitrogen from nutrient solutions applied at different pH values*

DATE OF TESTING.	MILLIGRAMS OF NITROGEN ABSORBED PER GRAM OF DRY MATTER PER HOUR					
	Ammonium series			Nitrate series		
	pH 4	pH 6	pH 8	pH 4	pH 6	pH 8
August 1	0 065	0 068	0 085	0 055	0 049	0 031
	0 061	0 075	0 089	0 052	0 046	0 043
Average	0 063	0 072	0 087	0 054	0 048	0 037
August 14	0 092	0 101	0 117	0 061	0 049	0 045
	0 081	0 095	0 132	0 055	0 045	0 039
	0 097	0 097	0 132	0 063	0 050	0 042
	0 090	0 110	0 119	0 058	0 046	0 039
Average	0 090	0 101	0 125	0 059	0 048	0 041
August 25		0 124	0 081	0 065	0 050	0 042
		0 115	0 075	0 055	0 038	0 040
		0 120	0 080	0 053	0 045	0 045
		0 101	0 078	0 059	0 048	0 043
Average		0 115	0 074	0 058	0 045	0 043

intermediate between the two in the  $\text{NH}_4$ -pH-8 treatment. From a comparison of these plant responses with nitrogen absorption rates in this series, it is apparent that in each of the three tests made, minimum nitrogen absorption rates occur in the  $\text{NH}_4$ -pH-4 treatments and correspond to minimum growth values, whereas maximum absorption rates occur in the  $\text{NH}_4$ -pH-8 treatments and correspond to intermediate growth values, maximum growth occurring with the intermediate absorption rates at pH 6.0. These relations hold for the first and second absorption tests, but in the third test, both maximum growth values and maximum absorption rates are shown for the  $\text{NH}_4$ -pH-6 treatments.

A similar comparison brings out the fact that in the nitrate series maximum, intermediate, and minimum growth values correspond to maximum, intermediate, and minimum nitrogen absorption rates, respectively. These occur in the  $\text{NO}_3\text{-pH-4}$ ,  $\text{NO}_3\text{-pH-6}$ , and  $\text{NO}_3\text{-pH-8}$  treatments, in the order given.

The data indicate clearly that peach trees absorb nitrogen as the cation from a culture solution at relatively high pH much more rapidly than they do from the same solution at relatively low pH; whereas they absorb nitrogen as the anion at a low pH of the culture solution at a much higher rate than they do at a high pH of the culture solution.

The relationships between growth and absorption, as here pointed out, appear to be fairly well defined in spite of the fact that the reaction change of the culture solutions, as they came in contact with the plant roots in passing through the sand cultures, was not adequately controlled, even when the solutions were renewed continuously at the rate of 4 liters per culture per day. It is to be emphasized, therefore, that, although these preliminary experiments yielded results which must be regarded as only approximate, they demonstrated that clearly defined differences in growth and distinct effects resulting from the treatments are to be expected if the reactions of the culture media are maintained within relatively narrow limits around the initial reactions. They have thus served as a guide for the 1932 experiments.

*Appearance of plants.* A qualitative criterion of considerable importance in judging the plants of these experiments was that of the general appearance. Striking differences occurred in the general appearances of the plants in the different groups of both series. In the ammonium series the leaves of the  $\text{NH}_4\text{-pH-4}$  plants remained dark green and were duller and smaller than those of normal peach trees. The tissues of these plants were also very hard and rigid. There were, however, no outstanding differences in appearance between the  $\text{NH}_4\text{-pH-6}$  and the  $\text{NH}_4\text{-pH-8}$  plants. This is what might have been expected, since throughout the period of the experiments there was little difference in the rates of growth of plants in these treatments.

In the nitrate series, differences in the appearance of the plants in the various treatments were quite obvious but not so striking as the differences in the ammonium series. The  $\text{NO}_3\text{-pH-4}$  plants had large, dark green leaves, whereas the leaves of the  $\text{NO}_3\text{-pH-8}$  plants were smaller and lighter green than those of other plants in the nitrate series. The  $\text{NO}_3\text{-pH-6}$  plants were usually intermediate in appearance between those of the pH-4 and pH-8 treatments, although they resembled closely the  $\text{NO}_3\text{-pH-4}$  plants.

An obvious feature in a comparison of the plants produced in the two series was the almost complete absence of any iron chlorosis in the ammonium series, as contrasted with the marked tendency of the  $\text{NO}_3\text{-pH-6}$  plants to become chlorotic—a characteristic which was still more pronounced in the  $\text{NO}_3\text{-pH-8}$  plants. This is in agreement with the results of others (7, 28, 29). It should be emphasized, however, that the slightest tendency toward chlorosis was

corrected quickly and that no differences in growth response among these plants can be attributed to chlorosis.

Aside from differences in height there were only relatively slight differences in appearance between plants in the pH-6 and pH-8 treatments of the ammonium series and those in the pH-4 and pH-6 treatments of the nitrate series. In figures 1 and 2 of plate 1 are shown typical plants from each series, photographed on August 13, the day before the second absorption test was made.

The response of the root systems of the plants to the reactions used in the two series was even more pronounced than the response of the tops. Thus, in the ammonium series, root growth in the pH-4 treatment was retarded considerably, and by August 14, when the second absorption test and harvest were made, there were many dead rootlets on these plants. Because of this, the pH-4 treatment of the ammonium series was discontinued. The  $\text{NH}_4$ -pH-6 plants usually produced roots that were somewhat longer than those of the  $\text{NH}_4$ -pH-8 plants. At the time of the second harvest, some of the roots on the  $\text{NH}_4$ -pH-6 plants showed some dead rootlets and some discoloration of most of the normally white portions of the root systems. This condition was undoubtedly the result of reaction change from the original pH of the culture solution to a point beyond the range of acid immunity in this treatment. This condition was avoided in later experiments by the use of a larger quantity of culture solution per day with more rapid rates of continuous solution renewal.

In the nitrate series, the best root development took place in the pH-4 treatment. In the pH-6 treatment the upper portions of the root systems produced few rootlets. In the pH-8 treatment of the nitrate series, practically no active root growth was made except at the extreme lower portion of the root systems.

Figures 1 and 2 of plate 2 show typical roots from the pH-6 and pH-8 treatments of the ammonium series, and the pH-4 and pH-6 treatments of the nitrate series, respectively, photographed after the last harvest and absorption test on August 25.

### *1932 experiments*

A group of 40 uniform peach seedling trees, about 25 cm. high, grown as previously described (1), were transplanted into twenty 2-gallon stoneware percolators prepared as in the preliminary experiments of 1931. From August 18 to September 12, all the plants were supplied, by the continuous renewal method, with solution  $\text{T}_3\text{R}_1\text{C}_3$ , of the modified Tottingham series of Jones and Shive (7), previously tested and known to be an excellent medium for the growth of peach trees. This was done in order to obtain a group of uniform plants of ample size to provide sufficient material for chemical analyses when the plants were harvested at the end of the experimental growth period.

On September 12, the final treatments were established. The 20 cultures of peach trees, now quite uniform in size and general appearance, were divided



into two groups of 10 cultures each. One group was used for the ammonium series, and of these, five cultures were treated with the solution indicated for this series in table 1, adjusted to pH 4, and the other five were treated with the solution adjusted to pH 6. The second group was used for the nitrate series, which was divided likewise into two sets of five cultures each and supplied with the solution indicated for this series in table 1, adjusted to pH values corresponding to those of the solution used in the ammonium series.

Attempts were made to maintain the reactions of the culture solutions in contact with the plant roots within a narrow range about the initial pH values of the respective solutions. Throughout most of the experimental period the reactions of the culture solutions were not altered more than 0.5 of a pH unit while percolating through the sand cultures. Each culture of the two series was supplied continuously with the culture solutions at the rate of 2 liters per day from September 12 to September 24. The rate of supply was then increased to 4 liters per day. On October 17, it became apparent that a still higher rate of supply would be required to maintain the pH of the solutions supplied to some of the cultures within the desired limits. Accordingly the rate of supply was increased to 6 liters per day and then to 8 liters per day which rate was maintained from October 21 to the end of the experimental period.

Because of the large quantities of nutrient solutions required daily after October 17, they were prepared in 90-liter glazed stoneware crocks. The leachings from the cultures were collected and returned to the crocks, where the solutions were readjusted to their initial pH value. These solutions were then used for a second day's supply.

Iron was supplied to the cultures by adding a solution of ferrous sulfate directly to the culture solutions at the rate of 0.2 to 0.4 p.p.m. of iron. Occasionally it was necessary to flush some of the pH-6 cultures of the nitrate series with 400 cc. of a 0.1 per cent solution of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ . Chlorosis was always controlled by this treatment.

*Growth responses and nitrogen absorption.* The peach trees of the 1932 experiments responded more definitely and with much more clearly defined relationships to the cultural treatments applied to them than did the trees in the 1931 experiment. This was undoubtedly due to the much higher rates of continuous solution renewal, which prevented excessive change in the reaction of the culture solution in contact with the plant roots while passing through the sand. However, the general trends and the results, in the main, were quite similar in the two sets of experiments.

Aside from the higher rates of solution renewal, the 1932 experiments were carried out by precisely the same methods as were the preceding ones. Growth responses were measured by the same criteria, and the nitrogen absorption tests were conducted in precisely the same manner in the two sets of experiments.

The nitrogen absorption rates of the plants in the cultures of the two series

were determined only once in connection with the 1932 experiments. These tests were conducted at the end of the experimental period, October 28 and 29, after which the plants were harvested and the green and dry weight yields obtained. The data relating to growth criteria are presented in table 4, and the results of the nitrogen absorption tests are recorded in table 5.

TABLE 4

*Total linear growth made during the experimental period, and green and dry yields per plant in the 1932 experiments*

	AMMONIUM SERIES		NITRATE SERIES	
	pH 4	pH 6	pH 4	pH 6
	cm.	cm.	cm.	cm.
Total linear growth. . . . .	132	403	450	306
	gm.	gm.	gm.	gm.
Green weight of plants:				
Stems . . . . .	12 63	35 02	42 26	27 33
Leaves . . . . .	24 58	67 02	72 85	50 68
Roots . . . . .	39 53	65 46	69 88	48 41
Whole plant . . . . .	76 74	167 50	184.99	126 42
Dry weight of plants:				
Stems . . . . .	5 03	11 36	14 29	7 92
Leaves . . . . .	6 62	16 79	18 79	11 09
Roots . . . . .	6 66	7 56	8 87	6 36
Whole plant . . . . .	18 31	35.71	41 95	25 37

TABLE 5

*Rates of absorption of ammonium and nitrate nitrogen, expressed as milligrams per gram of dry matter per hour by plants in the different treatments of the 1932 experiments*

AMMONIUM SERIES		NITRATE SERIES	
pH 4	pH 6	pH 4	pH 6
0.055	0.118	0.080	0.032
0.051	0.112	0.089	0.032
0.055	0.110	0.089	0.039
.....	0.121	0.083	0.032
.....	0.108	0.090	0.031
Average 0.052	0.114	0.086	0.033

The average dry weight yields in grams as given in table 4 and the average absorption rates in milligrams per gram of dry plant material per hour, are brought together for comparison and shown diagrammatically in figure 1. The bars on the left of the zero line represent the nitrate series; those on the right, the ammonium series. Inspection of this diagram shows clearly that in

the ammonium series the high average dry weight yields were produced in the pH-6 treatment and the low yields in the pH-4 treatment, and these are directly related to high and low rates of the absorption of nitrogen as the cation by the peach trees from the pH-6 and from the pH-4 solutions, respectively. In this series, plant measurement values and nitrogen absorption rate values for the trees in the pH-6 treatment are about double the corresponding values for those in the pH-4 treatment.

In the nitrate series, on the other hand, the high yields were produced in the pH-4 treatment and low yields in the pH-6 treatment, and these are directly related to high and low rates of the absorption of nitrogen as the anion by the trees from the pH-4 and the pH-6 solutions, respectively. In this series, plant measurement values of the trees in the pH-4 treatment are nearly double those

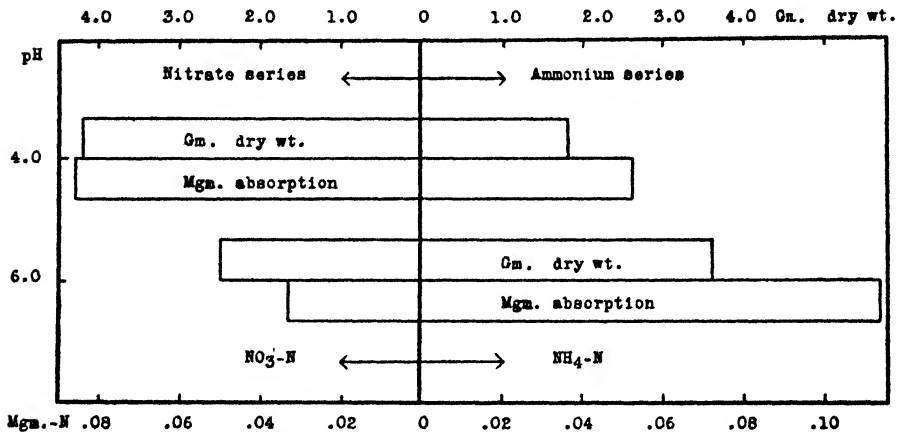


FIG. 1. AVERAGE DRY WEIGHT YIELDS IN GRAMS AND AVERAGE ABSORPTION RATES IN MILLIGRAMS PER GRAM OF DRY PLANT MATERIAL PER HOUR

of the trees in the pH-6 treatment, and the nitrogen absorption rate values for those in the former are nearly three times the corresponding values for those in the latter.

By comparing, now, the yield values and absorption rates for the trees in the ammonium series with those in the nitrate series, as indicated in figure 1, it will be observed that the high average dry weight per tree produced in the pH-6 treatment of the ammonium series is somewhat lower than that produced in the pH-4 treatment in the nitrate series. Similarly, the low dry weight yield produced in the pH-4 treatment of the ammonium series is considerably lower than that produced in the pH-6 treatment of the nitrate series.

On the other hand, cation-nitrogen absorption rates associated with high and low dry weight values in the ammonium series are considerably higher than anion-nitrogen absorption rates associated with high and low dry weight yields in the nitrate series. This means, of course, that the amount of plant material

produced in proportion to the nitrogen absorbed is much higher for the trees in the nitrate series than it is for those in the ammonium series.

As stated in connection with the preceding experiments, measurements were made of the increase in height of the main stem of each tree. From these measurements, recorded every 3 or 4 days, the average daily increment in height of the main stem per plant was calculated for the plants of each treatment in the two series throughout the experimental period. Since these values

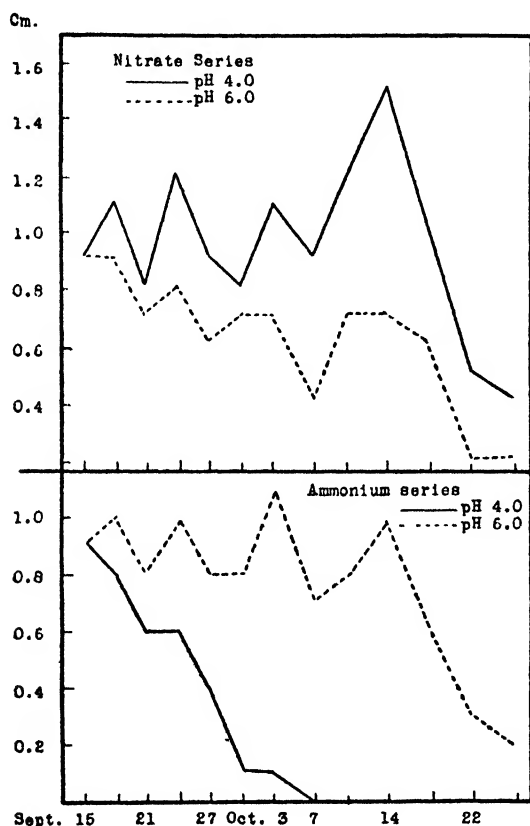


FIG. 2. AVERAGE GROWTH INCREMENTS AS INDICATED BY INCREASE IN HEIGHT OF MAIN STEMS PER DAY

are closely correlated with yields of plant substance, they indicate the trend of the influence of the pH treatments upon the growth of the peach trees over the experimental period from September 16 to October 26. The values of these average daily increments were plotted to form the two sets of graphs of figure 2, the upper set representing the nitrate series and the lower the ammonium series. These graphs clearly show, on the one hand, the complete superiority of the plants in the  $\text{NO}_3\text{-pH-4}$  treatment over those in the  $\text{NO}_3\text{-pH-6}$  treatment; and on the other hand, the complete superiority of the plants in the  $\text{NH}_4\text{-pH-6}$

treatment over those in the  $\text{NH}_4\text{-pH-4}$  treatment throughout the entire growth period. In fact, as indicated by the lower graph representing the ammonium series, the plants in the  $\text{NH}_4\text{-pH-4}$  treatment ceased to make any linear growth whatsoever after October 7.

*Appearance of plants.* In appearance the plants in these experiments were similar to those grown in the corresponding treatments in 1931, except that the characteristic differences resulting from the various treatments were more pronounced and much more clearly defined. There were, however, no significant differences in appearance between the  $\text{NH}_4\text{-pH-6}$  plants and the  $\text{NO}_3\text{-pH-4}$  plants except in gross size, and this became apparent only during the last 2 weeks of the experimental period. There was little difference in size or appearance between the roots produced by plants in the pH-4 cultures of the ammonium series and those in the pH-6 cultures of the nitrate series, except that the root systems of the former were very shallow and exhibited comparatively short rootlets; whereas those of the latter were poorly developed throughout the upper half of the root system but fairly well developed in the lower half. The root systems of the trees in the  $\text{NH}_4\text{-pH-6}$  cultures were more profusely branched into numerous fine rootlets than were those of trees in the pH-4 cultures of the nitrate series. There was, however, no noticeable difference in the gross size of the root systems produced in these two treatments.

In view of the fact that ammonia is one of the initial stages in nitrogen assimilation according to Eckerson (3), Prianischnikow (16), Robinson (19), and others, it is reasonable to assume that, if the proper conditions are supplied, ammonium nitrogen will be directly assimilated. Prianischnikow (18), Pirschle (13), and Tiedjens et al. (28, 29) have shown that under favorable conditions ammonium may be readily assimilated by higher plants, and that in many instances the growth of such plants, when supplied with ammonium compounds as the sole source of nitrogen, may exceed that made by the plants when grown with nitrates as a source of nitrogen.

The results of these experiments leave little doubt about the ability of peach trees to assimilate directly ammonium nitrogen. They show, moreover, that the growth of trees in balanced solutions containing nitrogen only in the ammonium form is closely dependent upon the reaction of the culture medium. Thus it is not enough to supply the culture solution at pH 6 in order to obtain good growth. It is also necessary to supply the solution at this reaction in such quantities as may be required to maintain the pH of the cultures within a favorable range, which, for the peach, appears to be relatively narrow, extending not much above nor much below pH 6.0. This is well illustrated by the superior growth made by the trees in the  $\text{NH}_4\text{-pH-6}$  treatment during 1932 as compared with that made by the trees in the corresponding treatment of the 1931 experiments. The difference was unquestionably due to the maintenance of a more favorable pH range in the cultures of the 1932 experiments. These results are in agreement with those found by others (12, 13, 28, 29) working with different species.

The very poor growth which occurred in the pH-4 treatment of the ammonium series was not duplicated in any treatment of the nitrate series. All cultures in the latter series appeared to support at least fair growth. This is an indication that the absorption and assimilation of nitrate nitrogen resulting in normal growth of the peach tree are somewhat less dependent upon the reaction of the culture solution than are the absorption and assimilation of ammonium nitrogen. Others (13, 28, 29) have likewise obtained good growth over a relatively wide range of reactions with nitrate treatments. Nevertheless, in the 1932 experiments in which the reaction of the  $\text{NO}_3$ -pH-6 cultures was maintained within a very narrow range around pH 6 during the growth period, the trees produced only about 60 per cent as much dry plant material as did those grown in the  $\text{NO}_3$ -pH-4 cultures.

*Plant analysis.* Immediately after the absorption tests were completed, the plants in both the ammonium and the nitrate series were harvested on October 30 and 31, respectively. The various nitrogenous fractions present in the stems and in the soft, white, fibrous roots were determined. A detailed description of the methods used for the analyses of the nitrogenous constituents of the plants has been presented in a previous publication (2). Unfortunately, however, the method for determining the cyanogenetic nitrogen fraction described in this publication was not available at the time the analyses were made of the plants grown in the 1932 experiments. In these analyses this fraction was represented by the loss of nitrogen during hydrolysis by boiling an aliquot of the soluble nitrogen extract for 2½ hours with 5 per cent sulfuric acid. Subsequent work has shown that this method accounted for only approximately 70 per cent of the cyanogenetic nitrogen present, and that the remaining 30 per cent of this fraction was included with the amide fraction. Corrections have been made for the amounts of cyanogenetic and amide nitrogen determined, and the corrected values for these fractions are included in the tables which follow. The results of the analyses are presented in tables 6 and 7.

In general, most of the nitrogenous fractions, as well as total nitrogen, were present in higher percentages in the stems of plants in the ammonium series than in those of the nitrate series. In proportion to the total organic nitrogen present in the stems, however, there was relatively little difference in the distribution of organic nitrogenous fractions among the plants in any except the  $\text{NH}_4$ -pH-4 treatment. The stems of plants in that treatment were relatively low in total amino and basic-free amino nitrogen, but they contained approximately twice as high a percentage of basic nitrogen as the stems of plants in any other treatment. The ammonium nitrogen was no higher in the stems of plants grown in the ammonium series than in those grown in the nitrate series. A small but quantitative amount of nitrate nitrogen was found in the stems of plants grown in the  $\text{NO}_3$ -pH-6 cultures, but no nitrates could be detected, even microchemically, in the stems of trees in any other treatment.

The fibrous roots of plants grown in the ammonium series contained con-

siderably higher proportions of soluble organic nitrogen and of all the soluble organic nitrogenous fractions than the roots of plants in the nitrate series. Roots of the former series also contained higher proportions of ammonium

TABLE 6

*Nitrogenous fractions in stems and roots of peach trees in ammonium series, expressed as percentage of green and dry matter, and as percentage of total organic nitrogen*

	pH-4 CULTURES			pH-6 CULTURES		
	Green weight basis	Dry weight basis	Total organic N as 100 per cent	Green weight basis	Dry weight basis	Total organic N as 100 per cent
<i>Stems</i>						
Total N. . . . .	0 684	1 718		0 501	1 524	
Total organic N. . . . .	0 673	1 692	100 00	0 486	1 479	100.00
Protein N. . . . .	0 322	0 808	47 77	0 266	0 808	54 61
Soluble organic N. . . . .	0 351	0 884	52 23	0 220	0 671	45.39
Cyanogenetic N. . . . .	0 023	0 058	3 42	0 008	0 026	2 93
Amide N. . . . .	0 073	0 184	10 87	0 060	0 182	12 29
Humin N. . . . .	0 017	0 041	2 55	0 012	0 038	2.51
Total amino N. . . . .	0 120	0 302	17 86	0 102	0 311	20 93
Basic-free amino N. . . . .	0 075	0 188	11 08	0 074	0 226	15.25
Basic N. . . . .	0 154	0 388	22.91	0 052	0 160	10.77
Ammonium N. . . . .	0 011	0 027	*1 55	0 015	0 045	*2 91
Nitrate N. . . . .						
Other N. . . . .	0 009	0 025	1 40	0 014	0 039	1 64
<i>Fibrous roots</i>						
Total N. . . . .	0 404	3 931		0 287	3 694	
Total organic N. . . . .	0 393	3 828	100 00	0 272	3 504	100 00
Protein N. . . . .	0 262	2 547	66 55	0 140	1 802	51 43
Soluble organic N. . . . .	0 131	1.281	33 45	0 132	1 702	48.57
Cyanogenetic N. . . . .	0 017	0 169	4 42	0 005	0 070	1.99
Amide N. . . . .	0 035	0 337	8 80	0 030	0 389	11 11
Humin N. . . . .	0 008	0.074	1 93	0.009	0.132	3.31
Total amino N. . . . .	0 049	0 477	12 46	0.056	0.720	20 53
Basic-free amino N. . . . .	0 031	0.308	8 03	0 034	0 440	12.58
Basic N. . . . .	0 036	0.350	9 15	0 041	0 529	15 08
Ammonium N. . . . .	0 011	0 103	*2 62	0 013	0.168	*4 54
Nitrate N. . . . .				0.002	0.023	*0.63
Other N. . . . .	0 004	0 043	1.12	0 013	0 142	4.45

\* Percentage of total nitrogen.

nitrogen than did those in the nitrate series. The one outstanding difference in composition among the roots grown in the two series is the very high proportion of inorganic nitrogen present as nitrates in the roots of plants grown in the nitrate series.

*The assimilation of nitrogen and the distribution of nitrogen fractions.* Chemical analyses of the plants in the ammonium and nitrate series show some interesting relationships between growth and composition. The most striking dif-

TABLE 7

*Nitrogenous fractions in stems and roots of peach trees in nitrate series, expressed as percentage of green and dry matter, and as percentage of total organic nitrogen*

	pH-4 CULTURES			pH-6 CULTURES		
	Green weight basis	Dry weight basis	Total organic N as 100 per cent	Green weight basis	Dry weight basis	Total organic N as 100 per cent
<i>Stems</i>						
Total N. . . . .	0 437	1.267		0 418	1 281	
Total organic N . . . . .	0 422	1 226	100 00	0 393	1 203	100 00
Protein N. . . . .	0 219	0 636	51 85	0 218	0 666	55 39
Soluble organic N. . . . .	0 203	0 590	48 15	0 175	0 537	44 61
Cyanogenetic N. . . . .	0 013	0 039	3 08	0 014	0 042	3 46
Amide N. . . . .	0 050	0 146	11 94	0 045	0 138	11.46
Humic N. . . . .	0 012	0 033	2 65	0 013	0 040	3 36
Total amino N. . . . .	0 083	0 241	19 72	0 077	0 237	19 66
Basic-free amino N. . . . .	0 062	0 181	14 79	0 057	0 173	14 41
Basic N. . . . .	0 047	0 135	11 00	0 047	0 143	11 92
Ammonium N. . . . .	0 014	0 041	*3 25	0 012	0 037	*2.89
Nitrate N. . . . .				0 013	0 041	*3 20
Other N. . . . .	0 019	0 056	4 69	0 001	0 001	.....
<i>Fibrous roots</i>						
Total N. . . . .	0 283	3 974		0 284	3 675	
Total organic N. . . . .	0 101	1 420	100 00	0 208	2 692	100 00
Protein N. . . . .	0 035	0 487	34 24	0 154	1.992	74 04
Soluble organic N. . . . .	0 066	0 933	65 72	0 054	0 700	25 96
Cyanogenetic N. . . . .	0 004	0 059	4 15	0 005	0 062	2 31
Amide N. . . . .	0 016	0 222	15 63	0 014	0 186	6.92
Humic N. . . . .	0 003	0 041	2 87	0 001	0 016	0 58
Total amino N. . . . .	0 038	0 536	37 75	0 028	0 357	13.27
Basic-free amino N. . . . .	0 022	0 303	21 35	0 015	0 197	7 31
Basic N. . . . .	0.021	0 298	20 95	0 017	0 217	8 08
Ammonium N. . . . .	0 002	0 034	*0 85	0 003	0 039	*1.06
Nitrate N. . . . .	0 180	2 520	*63 42	0 073	0 944	*25.67
Other N. . . . .	.....	0.010	0 77	0.002	0 022	0.76

\* Percentage of total nitrogen.

ferences in composition occurred in the roots of the two series. This should be expected, however, since the roots are the organs of absorption and, in the case of the peach tree, the chief organs for the initial stages of nitrogen assimilation.



The stems of the trees grown in the ammonium series contained from 19 to 35 per cent more nitrogen than did those of the trees grown in the corresponding cultures of the nitrate series, as shown in tables 6 and 7.

The  $\text{NH}_4\text{-pH-4}$  plants contained higher proportions of nitrogen in stems and roots than did the  $\text{NH}_4\text{-pH-6}$  plants. This greater yield in total nitrogen in the stems of the plants of this series can be accounted for entirely by the very high proportion of basic nitrogen found in stems of the  $\text{NH}_4\text{-pH-4}$  plants as compared with that found in stems of the  $\text{NH}_4\text{-pH-6}$  plants. In the roots, however, the greater yield of total nitrogen was due to a very high proportion of protein nitrogen.

No significant differences were found in the percentages of nitrogen contained in the stems of plants in the nitrate series.

Tiedjens and Blake (28) found that high yields of dry matter in apple roots were associated with high proportions of total nitrogen. This relation was found to hold for the peach roots grown in the ammonium series, but it did not apply in the case of roots grown in the nitrate series. A direct relation was found in both series, however, when the percentage of total organic nitrogen was compared with the percentage of dry matter in the roots. Total nitrogen in peach roots grown in nitrate cultures can have little relation to assimilation, since the roots may contain very large proportions of inorganic nitrogen as nitrates.

*Inorganic nitrogen.* The inorganic nitrogen present in the peach roots bears a direct relation to the rate of absorption of nitrogen and to growth. This relation is shown graphically in figure 3, which indicates that, when supplied with an ammonium solution at pH 6, peach roots absorbed nitrogen at a much higher rate and that they contained considerably more ammonium nitrogen than when they were provided with a similar solution adjusted to pH 4. Likewise it is observed in the nitrate series that the roots both absorbed nitrate nitrogen more rapidly and contained a higher percentage of nitrate nitrogen when the culture solution was supplied at pH 4 than when it was supplied at pH 6.

The fact that more than half of the total nitrogen in the absorbing roots of peach trees may exist as nitrates while no nitrates are found in the tops, is a very good indication that amino acid formation in the peach takes place in the roots, just as Thomas (27) found that this process takes place in the roots of the apple tree. Furthermore, since the concentration of ammonium nitrogen was low in the stems of the peach trees in both the ammonium and the nitrate series, and was no higher in the trees of the former than in those of the latter, the indication is that ammonium nitrogen is assimilated likewise mainly in the roots.

It was observed that toward the end of the growing season nitrates usually accumulated in the stems, of peach trees grown in  $\text{NO}_3\text{-pH-6}$  cultures but were absent from the stems of the  $\text{NO}_3\text{-pH-4}$  plants. This accumulation of nitrates in the tops, together with the growth responses of these plants, indicates a lack

of ability on the part of the plants to assimilate this form of nitrogen efficiently when grown in cultures with relatively high pH values.

These data are very interesting when studied in comparison with the findings of Emmert (4), who reports as much as 600 p.p.m. of nitrates in the petioles of peach trees grown in the field. It is seen that under the conditions furnished in the  $\text{NO}_3$ -pH-6 treatment, nitrates accumulated in the stems of the peach trees in concentrations of 130 p.p.m. of nitrogen or 570 p.p.m. of nitrates. The samples with which these determinations were made included a large proportion of wood in which there was no nitrate nitrogen. It is reasonable to assume, therefore, that in the more active and less woody portions of the

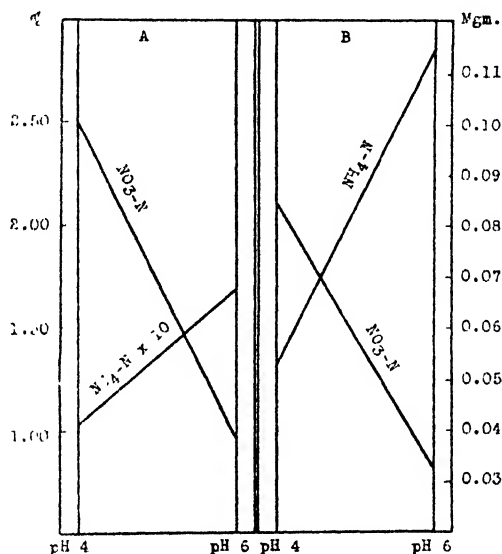


FIG. 3. A, INORGANIC NITROGEN IN PEACH ROOTS EXPRESSED IN PER CENT OF DRY MATTER. B. RATES OF ABSORPTION OF INORGANIC NITROGEN BY PEACH TREES EXPRESSED IN MILLIGRAMS PER GRAM OF DRY MATTER PER HOUR

stems, the concentrations of nitrates were much higher. One may anticipate, therefore, that nitrates may accumulate in the above ground parts of peach trees grown on heavily limed soil having a high pH value and an abundance of nitrogen in the nitrate form.

**Total organic nitrogen.** This fraction is composed of protein nitrogen and the various soluble organic nitrogenous constituents. The relation of total organic nitrogen to the other organic nitrogenous fractions in stems and roots of trees in both series is shown graphically in figures 4 and 5. The variations in this fraction among the different treatments have been considered in connection with total nitrogen. It may be observed (fig. 5) however, that in the roots of the  $\text{NO}_3$ -pH-4 cultures the concentration of total organic nitrogen was very low. Of the total nitrogen in these roots, 63.42 per cent was in

the nitrate form. Nevertheless, this treatment supported the most rapid growth that occurred in any culture. This appears to indicate that nitrogen was transported from the absorbing roots to other parts of the  $\text{NO}_3\text{-pH-4}$  plants only as rapidly as it was elaborated.

*Protein nitrogen.* In general, the protein content was relatively high in those plants which grew slowly and produced low yields of dry plant substance. The proportions of protein nitrogen present in the stems and roots of trees grown in the several pH treatments are shown diagrammatically in figures

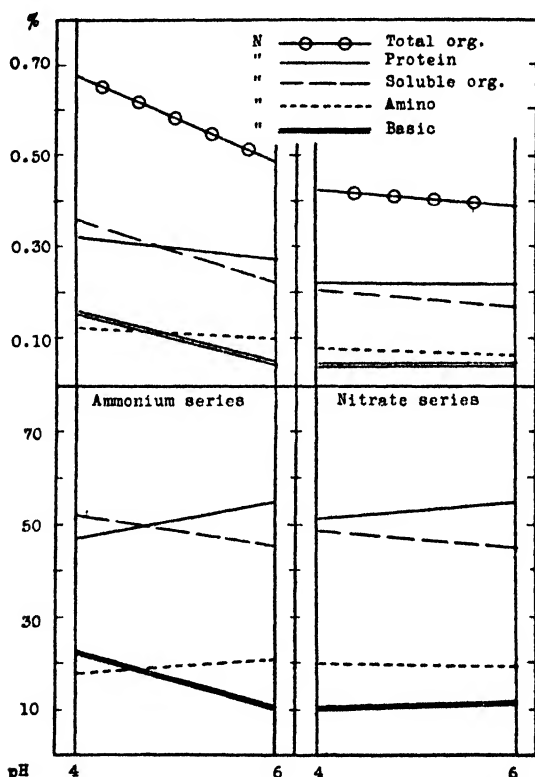


FIG. 4. NITROGENOUS FRACTIONS IN STEMS OF PEACH TREES EXPRESSED IN PER CENT GREEN MATTER (ABOVE), AND IN PER CENT TOTAL ORGANIC NITROGEN (BELOW)

4 and 5. These indicate clearly that the low yields of dry plant material made by the  $\text{NH}_4\text{-pH-4}$  plants were associated with a high proportion of protein nitrogen in their stems and roots, whereas the high yields made by the  $\text{NH}_4\text{-pH-6}$  plants were associated with a relatively low proportion of protein nitrogen in both stems and roots. This is in agreement with the work of Sessions and Shive (21). By a similar manner of comparison, the trees in the  $\text{NO}_3\text{-pH-6}$  cultures, which showed lower yields of dry matter than those in the  $\text{NO}_3\text{-pH-4}$  cultures, contained relatively higher proportions of protein nitrogen than were found in the latter plants.

**Soluble organic nitrogen.** The soluble organic nitrogen found in the peach trees grown in the 1932 experiment was composed mainly of amide, amino, and basic nitrogen. The analyses show that, with the exception of the stems of the  $\text{NH}_4$ -pH-4 plants a relatively high proportion of soluble organic nitrogen was always associated with high yields of dry plant substance in each of the two series.

**Cyanogenetic nitrogen.** There appears to be no obvious relation between the content of cyanogenetic nitrogen and the growth responses of the trees

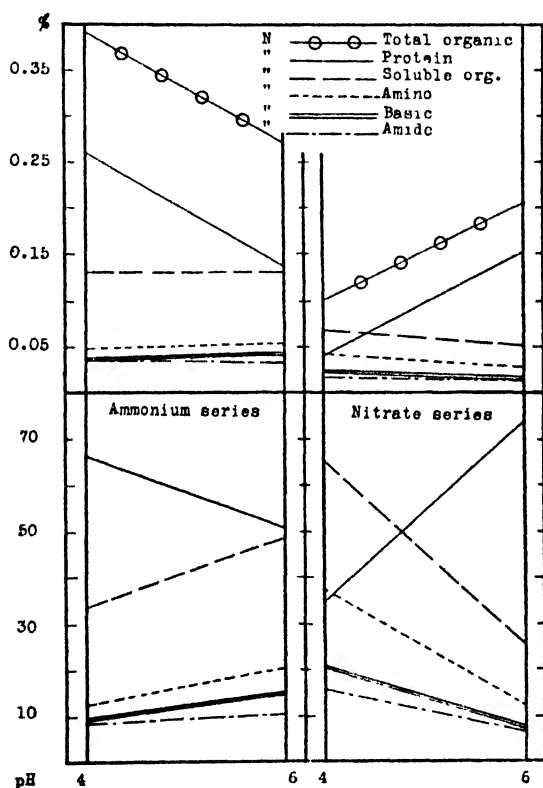


FIG. 5. NITROGENOUS FRACTIONS IN PEACH ROOTS EXPRESSED IN PER CENT GREEN MATTER (ABOVE), AND IN PER CENT TOTAL ORGANIC NITROGEN (BELOW)

or yield values in these experiments. It is probable that this form of nitrogen may serve as a quickly available reserve nitrogenous fraction, although there was no clearly defined evidence to indicate that it served such a function in these experiments. Robinson (20) has shown that this fraction is subject to wide seasonal variations, and that, at times, it may represent a major nitrogenous fraction.

**Amide nitrogen.** Suzuki (25, 26) believed that amides serve as intermediate products in the synthesis of proteins from nitrates. Later Prianischnikow (14)

claimed that amides are an intermediate form of nitrogen both in the synthesis of protein from ammonia and in the hydrolysis of protein to ammonia. Prianschnikow (15) also found that amide nitrogen was formed more rapidly in *Vicia sativa* and in *Zea mays* seedlings when supplied with ammonium salts together with calcium carbonate than when calcium nitrate served as the source of nitrogen in the growth medium. Nightingale (10, 11) found that amides increase at the expense of proteins when plants are kept in darkness, showing that amides are certainly intermediate nitrogenous compounds formed during proteolysis. The results of Tiedjens and Blake (28) confirmed those of Prianschnikow (15) in that they found considerably higher concentrations of amides in the roots of apple trees grown in ammonium cultures than they found in roots from nitrate cultures. In still later work, Prianschnikow (16) found that a high degree of acidity in a nutrient solution hindered the formation of amides in plants. Mevius and Engle's (8) work did not support Prianschnikow's results. Likewise Tiedjens and Blake (28) did not find any more amides in the roots of apple trees grown in pH-6 cultures than in pH-4.5 cultures, although amides did increase at higher pH values of the culture medium.

The amide form of nitrogen was relatively high in the peach trees grown in these experiments. It did not, however, vary a great deal among the trees grown in the cultures of the two series. In both stems and roots, amides always represented a larger proportion of the total organic nitrogen in the plants of that treatment which in each series supported high yields of plant substance than they represented in plants grown in the treatment that supported low yields. This was particularly true in the roots of trees grown in the nitrate series, since the  $\text{NO}_3$ -pH-4 roots contained more than twice as much amide nitrogen in proportion to the total organic nitrogen present as did the  $\text{NO}_3$ -pH-6 roots. The content of amides in proportion to the content of other nitrogenous fractions present in the roots of the peach trees grown in these experiments is shown diagrammatically in figure 5.

Although these results support Prianschnikow's (16) views, it is doubtful whether the poor growth made by the trees in the  $\text{NH}_4$ -pH-4 treatment resulted from an inadequate synthesis of amides. This expression of doubt is warranted since the  $\text{NH}_4$ -pH-4 plants actually contained a much higher percentage of amide nitrogen, although it was less in proportion to the other nitrogenous fractions than was found in the  $\text{NO}_3$ -pH-4 plants.

**Humin nitrogen.** According to Holme and Gortner (6), the acid-insoluble humin fraction is probably formed from tryptophane. It therefore represents one of the more complex soluble organic nitrogenous constituents of the peach. Tiedjens and Blake (28) found that humin nitrogen in the roots of apple trees was more closely associated with the growth of the trees than were the amino and amide fractions. Humin nitrogen usually represented the smallest organic nitrogenous fraction found in either the stems or the roots of the trees produced in the two series of these experiments. Nevertheless, the highest percentage

value of this fraction was directly associated with the highest yield values of plant substance in each of the two series.

*Amino nitrogen.* Although amino acids represent assimilated nitrogen in plants, they do not represent the final stages of assimilation. They probably serve as the chief forms of transportable, elaborated nitrogen in higher plants. In the peach trees of these experiments, as Thomas (27) found in the apple, inorganic nitrogen is probably elaborated largely to the amino form in the roots, and is then translocated to other parts of the plant. Considerable evidence is available in support of this suggestion.

It will be observed in tables 6 and 7 that the total amino nitrogen, expressed on a dry weight basis, was always highest in the stems and roots of trees in that treatment which, in each of the two series, supported the best growth. Thus a direct relation is shown between the content of amino nitrogen in the plants and their growth responses.

The same relation also holds for basic-free amino nitrogen and growth. The significance of the variations between total and basic-free amino nitrogen in the various treatments, however, is not clear.

These experiments support the findings of Tiedjens et al. (28, 29) that plants grown in media with ammonium as the source of nitrogen contain a higher percentage of amino nitrogen than do plants grown correspondingly in media with nitrate as the source of nitrogen.

*Basic nitrogen.* As determined in these analyses, basic nitrogen supposedly includes the basic amino acids and some peptide nitrogen. The fraction is therefore composed of somewhat more complex forms than the simple amino acids. With this in mind, it is interesting to study this fraction in the roots and stems of plants grown in the different treatments.

Invariably the stems of plants which made the best growth in each of the two series contained the lowest percentage of basic nitrogen, whereas the roots of the same plants contained the highest percentage of basic nitrogen. Thus the  $\text{NH}_4\text{-pH-6}$  plants contained a lower percentage of basic nitrogen in their stems but a higher percentage in their roots than was found in the  $\text{NH}_4\text{-pH-4}$  plants. Similarly, the  $\text{NO}_3\text{-pH-4}$  plants contained a lower percentage of basic nitrogen in the stems but a higher percentage in the roots than did the  $\text{NO}_3\text{-pH-6}$  plants.

The condition that existed in stems of the  $\text{NH}_4\text{-pH-4}$  plants is particularly interesting. It was mentioned previously that these plants were relatively low in protein nitrogen for slowly growing plants. Moreover, this condition was associated with a very high concentration of basic nitrogen. Also, the amino nitrogen content of these stems was probably no lower in comparison with that found in the  $\text{NH}_4\text{-pH-6}$  stems than the growth responses of the plants would indicate. Since the basic fraction may include considerable peptide nitrogen, it would seem to indicate that protein synthesis in these stems was probably being retarded in the peptide stage.

## SUMMARY

Peach trees were grown in sand cultures in two series of treatments, in one of which the cultures received nitrogen only in the ammonium form; in the other, they received nitrogen only in the nitrate form. The nutrient solutions in both series of treatments were applied at pH 4, 6, and 8. Absorption tests were conducted to determine the rates, in milligrams per gram of dry plant material per hour, at which nitrogen was absorbed by plants in the various treatments. Quantitative analyses were made to determine the relative distribution of various nitrogenous fractions in the plants grown in the treatments. The more important results may be summarized briefly as follows:

Peach trees made much better growth in sand cultures supplied continuously with a culture solution at pH 6 than at pH 4 or pH 8, with  $\text{NH}_4$  as the sole source of nitrogen.

Peach trees made much better growth in sand cultures supplied continuously with a culture solution at pH 4 than at pH 6 or pH 8 with  $\text{NO}_3$  as the sole source of nitrogen.

The trees grew about equally well in sand cultures at pH 6 with ammonium as the source of nitrogen and at pH 4 with nitrate as the source of nitrogen.

Ammonium nitrogen was absorbed at higher rates by trees supplied with solutions adjusted to pH 6 than by those supplied with solutions adjusted to pH 4.

Nitrate nitrogen was always absorbed at higher rates by trees supplied with solutions adjusted to pH 4 than by those supplied with solutions adjusted to pH 6 or 8.

At favorable pH values of the culture solutions, peach trees absorbed nitrogen at relatively higher rates when it was supplied in the ammonium form than when it was supplied in the nitrate form.

The initial stages of ammonium assimilation by the peach take place in the roots.

Nitrate reduction by peach trees takes place almost entirely in the roots.

Good growth and high yields of plant substance were usually associated with relatively low percentages of protein nitrogen, both in stems and in roots.

Good growth and high yields of plant substance were associated with relatively high percentages of amide, amino, and humin nitrogenous fractions.

Invariably the stems of trees that made the best growth and produced highest yields of plant substance were relatively low in basic nitrogen, while the roots of the same plants were relatively high in this fraction.

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## PLATE 1

FIG. 1. Representative trees grown in the ammonium series. Left,  $\text{NH}_4$ -pH-4 culture; right,  $\text{NH}_4$ -pH-6 culture.

FIG. 2. Representative trees grown in the nitrate series. Left,  $\text{NO}_3$ -pH-4 culture; right,  $\text{NO}_3$ -pH-6 culture.



FIG. 1



FIG. 2

## PLATE 2

FIG. 1. Roots of representative trees grown in the ammonium series. Left,  $\text{NH}_4$  pH 6 culture, right,  $\text{NH}_4$  pH 8 culture.

FIG. 2. Roots of representative trees grown in the nitrate series. Left,  $\text{NO}_3$  pH 4 culture, right,  $\text{NO}_3$  pH 6 culture.

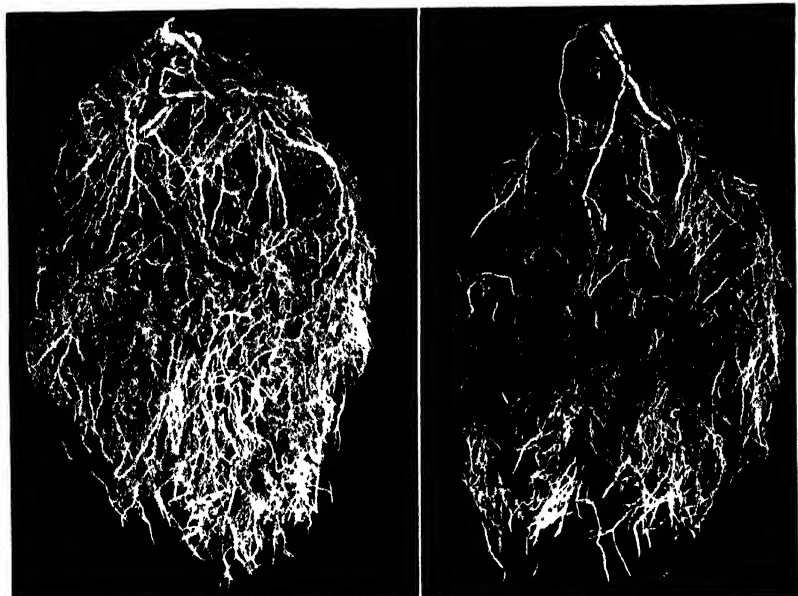


FIG. 1

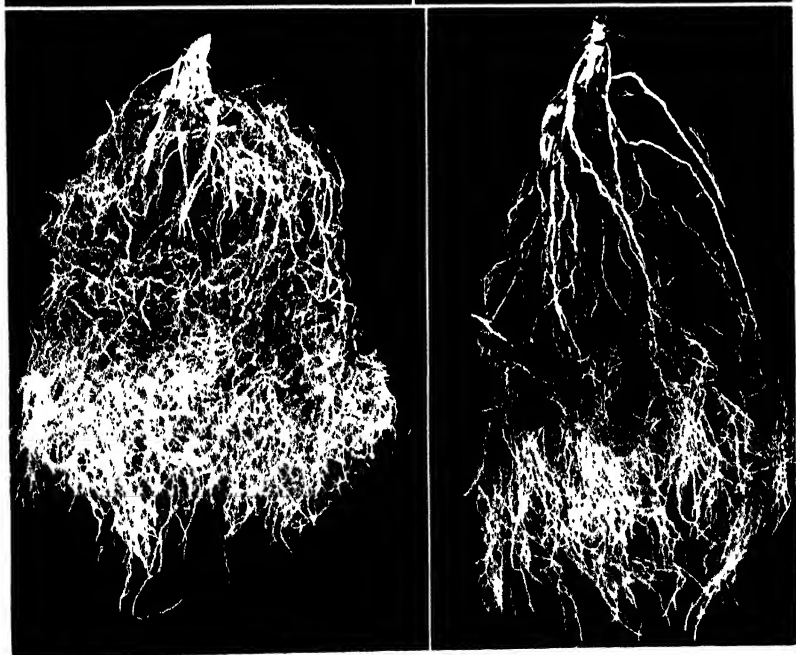


FIG. 2



# PHYSIOLOGICAL STUDIES ON RHIZOBIUM: I. THE EFFECT OF NITROGEN SOURCE ON OXYGEN CONSUMPTION BY RHIZOBIUM LEGUMINOSARUM FRANK<sup>1</sup>

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The discovery of the symbiotic relationship between leguminous plants and the root-nodule bacteria quite naturally led to the assumption that as the bacteria are the active agents in the fixation of nitrogen by inoculated legumes they should be able to fix nitrogen non-symbiotically when cultured in a suitable medium. The early work of Beijerinck (2) indicated that this was actually the case, although he did not find appreciable amounts of nitrogen fixed in his cultures. Later workers were more definite in concluding that the bacteria are capable of fixing nitrogen in the absence of the legume plant.

The work of a number of investigators in recent years, however, has thrown considerable doubt upon this assumption. The studies of Hopkins (10), Pohlman (13), Allison (1), Löhnis (11), and Wilson, Hopkins, and Fred (19) have been outstanding in this connection. All of these investigators came to the conclusion that under the conditions of their experiments, which were extremely variable, there was not a significant amount of nitrogen fixed by the organisms when grown alone. It has even been shown in Burk's (6) respiration studies with *Rhizobium*, that the organisms would not grow in a medium devoid of nitrogen. When ammonium or nitrate nitrogen was added growth occurred, as indicated by the consumption of oxygen by the organisms. Other investigators also have shown the desirability of adding small amounts of nitrogen to media in which the organisms are to be grown. Water extracts of leguminous plants, yeast extract, asparagin, nitrate, ammonium, and other forms of nitrogen have been used for this purpose.

Pohlman (13), although he obtained negative results, suggests that if proper environmental conditions are supplied, the root-nodule bacteria may be able to fix nitrogen when grown alone. After reviewing the literature on this subject Fred, Baldwin, and McCoy (8) concluded that "It is at least possible that sometime a new medium or new conditions of experiment may be found which will allow fixation." Although it seems that practically every known combination of conditions has been tried by the various investigators of this problem, there are still possibilities. As an example of what may be done, the recent work of Burk (7) may be cited. Until very recently it was thought absolutely

<sup>1</sup> Journal paper no. J 118 of the Iowa Agricultural Experiment Station. Project No. 226.

necessary to provide strictly aerobic conditions for the growth of *Azotobacter*, but by applying the theory of biological oxidation and reduction, as developed by Weiland and described by Buchanan and Fulmer (5), Burk has succeeded in growing *Azotobacter* under strictly anaerobic conditions, simply by furnishing the organisms with a suitable hydrogen acceptor in place of the free oxygen available under aerobic conditions. Although this work has no direct bearing upon the problem of nitrogen fixation by *Rhizobium*, it does, along with the recent work of Winogradsky (20) and of Blom (3), suggest that there is a possibility of providing a certain set of conditions under which the organisms would use free nitrogen as the hydrogen acceptor in the oxidation of carbohydrates for growth energy. What these conditions might be, remains to be determined, and investigational work along this line will certainly be worthwhile.

The whole problem of the nitrogen nutrition and metabolism of these bacteria is involved, and studies along these lines would undoubtedly contribute much toward an understanding of the process of nitrogen fixation as it is effected by the microorganisms. The results of such work would not only be of considerable scientific interest but they would have an influence upon the practice of soil and seed inoculation. Studies on the nitrogen metabolism of the bacteria of the genus *Rhizobium* have been outlined, therefore, and the present paper is a report of one phase of this work. It deals with the effect of various sources of nitrogen on the consumption of oxygen by *Rhizobium leguminosarum* Frank, the type species of the genus.

#### EXPERIMENTAL

Various procedures suggest themselves for studying the effects of free nitrogen and of various kinds of combined nitrogen on the metabolism of *Rhizobium*. The organisms may be grown on the surface of agar containing various nitrogen compounds and observations made of growth by the rapidity and extent of colony formation. This may be considered a qualitative test. Liquid cultures in the proper media may be used and comparative amounts of growth determined by turbidity methods. This is also a qualitative method. To obtain results of a quantitative nature, the numbers of organisms in liquid cultures at various intervals might be determined and the generation time, which is a good measure of rate of growth, calculated. This method was used in previous studies on *Rhizobium* by Walker, Anderson, and Brown (16).

In order to simplify the procedure, however, and at the same time obtain a more delicate measure of the effects of nitrogen on the metabolism of *Rhizobium*, new methods were sought. After considerable investigation respiration methods, used rather widely by the physiologists and to some extent by bacteriologists, were adopted. The particular apparatus and technic employed were developed by Warburg (17) and will be described briefly.

*Method*

Otto Warburg and his associates in Germany have been engaged for some years in a study of the metabolism of cells, particularly cells of tumors and cancer tissue, by the respiration method using specially devised respiration chambers attached to micromanometers. By this means it was possible to measure minute amounts of oxygen consumed and carbon dioxide produced by the cells. Essentially the same technic has been used extensively by Crozier at Harvard and by Burk at the Bureau of Chemistry and Soils.

The principal apparatus employed is a specially constructed vessel in which the organisms are grown, and to which a "U"-shaped manometer of capillary bore is attached by means of a ground glass joint. These are shown in figures 1 and 2 of plate 1.

Inside the culture vessel there is sealed a glass cup which holds an alkaline solution for absorbing the carbon dioxide produced by the organisms. In these experiments 0.3 cc. of 2 *N* KOH was employed for this purpose. The culture of organisms (2 cc.) is placed in the flask. As the organisms respire they consume oxygen from the atmosphere within the flask, inasmuch as they are aerobic. If the stopcock at the top of the manometer arm is closed to prevent contact between the outside atmosphere and the atmosphere within the flask, there will be a negative pressure developed in the flask as the organisms consume oxygen. This change in pressure is indicated by the level of the confining fluid within the manometer. Brodie's solution, because of its low specific gravity, is well adapted for use as this confining liquid. It has a standard pressure of 10,000 mm. compared with 760 mm. for mercury. It flows through the capillary of the manometer with ease and does not adhere to the sides of the tube. It may be prepared by dissolving 23 gm. of sodium chloride and 5 gm. of sodium choleate in 500 cc. of water and adding one drop of a concentrated alcoholic solution of thymol as a preservative. As the solution is colorless, it has been found desirable to add a little dye, such as neutral red, to facilitate the reading of the manometer.

The amount of oxygen consumed by the organisms may be calculated from the changes in pressure by use of the following equation developed by Warburg (17):

$$X = h \left( \frac{V_o - \frac{273}{T} + V_f \alpha_{O_2}}{P_o} \right)$$

where  $X$  = oxygen consumed in cubic millimeters,  $h$  = change in pressure at constant volume in millimeters of manometer fluid (Brodie's fluid),  $V_o$  = volume of gas space in cubic millimeters between the surface of liquid medium and the meniscus of the confining fluid in the manometer,  $V_f$  = volume of culture medium in cubic millimeters,  $\alpha_{O_2}$  = the solubility coefficient (Bunsen's coefficient) of oxygen in the medium,  $P_o$  = standard pressure in millimeters of manom-



eter fluid (10,000 mm. Brodie = 760 mm. Hg). For any given vessel at constant temperature and with constant  $V$ , the term within the bracket is a constant. Thus the change in height of the manometer fluid,  $h$ , multiplied by the vessel constant gives the cubic millimeters of oxygen consumed.

In the oxygen consumption experiments reported here a series of seven manometers were employed. The culture vessels were kept in a large water bath with the temperature thermostatically controlled at  $28^{\circ} \pm 0.01^{\circ}\text{C}$ . Figure 3 of plate 1 shows the manometers and the water bath as used in the experiments.

In all experiments one manometer and one vessel were used as a thermobarometer to serve as a control against changes in barometric pressure and minor changes in temperature. This was found to be very desirable since small fluctuations in barometric pressure may make an appreciable change in the level of the Brodie's solution and yet the change in barometric pressure would be so small that it would be imperceptible by the ordinary mercury barometer. Furthermore, the corrections of gas volumes for changes in atmospheric pressure were greatly facilitated by the use of one manometer as a thermobarometer.

### Preparation of cultures

A basic nitrogen-free medium of the following composition was prepared and used in all the experiments:

Glucose.....	10.0 gm.
$\text{K}_2\text{HPO}_4$ .....	0.5 gm.
$\text{MgSO}_4$ .....	0.2 gm.
$\text{NaCl}$ .....	0.1 gm.
$\text{CaCl}_2$ .....	0.1 gm.
Distilled water.....	1,000.0 cc.

In the various experiments different materials were added to this basic medium as sources of nitrogen. Ammonium chloride, sodium nitrate, urea, d-l-alanine and bacto-yeast-extract were employed as sources of nitrogen, and the organisms were grown in various concentrations of each of these materials. The concentrations of nitrogen in the culture media of different tests were as follows: none, 0.72, 7.2, 36.0, 72.0, and 144.0 p.p.m. of solution.

The organisms used in the experiments were rapidly growing cultures of the root-nodule bacteria of garden peas, *Rhizobium leguminosarum*. The inoculum was prepared by first growing the organisms on a large slant of yeast-mannitol agar containing 0.5 gm. of bacto-yeast-extract per liter. To prevent transfer of appreciable quantities of nitrogen from the yeast-mannitol slant the organisms were washed off with a small amount of sterile distilled water. One cubic centimeter of the resulting suspension of organisms was placed into the manometer vessel to which had been added 1 cc. of the sterile medium (double strength) in which the organisms were to be grown. The 1 cc. of inoculum for

each manometer vessel was taken from the same suspension of organisms for any one experiment to insure uniform inoculation of all vessels. After the culture had come to the temperature of the water bath and sufficient time had elapsed to permit complete absorption of the carbon dioxide of the atmosphere within the manometer vessel, the manometer stopcock was closed and the experiment started. In each case the tests were conducted over a period of 30 hours; changes in the level of the manometer fluid being observed at intervals of 1, 2, or 3 hours.

## RESULTS

In the first test a culture of *Rhizobium leguminosarum* was grown in the basic medium described earlier, containing various concentrations of nitrogen,

TABLE 1

*Oxygen consumption by Rhizobium leguminosarum in media containing yeast extract*

TIME	OXYGEN CONSUMPTION BY CULTURE					
	No nitrogen	Amounts of nitrogen as yeast extract in media				
		0.72 p.p.m.	7.2 p.p.m.	36.0 p.p.m.	72.0 p.p.m.	144.0 p.p.m.
hrs.	c.mm.	c.mm.	c.mm.	c.mm.	c.mm.	c.mm.
0	0.0	0.0	0.0	0.0	0.0	0.0
1	6.4	7.7	10.6	10.6	11.9	11.1
3	16.6	18.5	29.0	30.4	36.4	35.3
5	27.9	32.5	52.6	58.3	68.8	67.5
7	38.4	45.9	76.2	93.7	109.4	113.0
9	48.5	59.3	100.2	138.5	164.0	173.5
11	57.4	71.2	120.0	186.6	223.5	245.9
12	61.4	76.8	131.3	213.8	258.2	288.7
14	72.3	87.9	149.0	261.9	317.0	364.2
16	79.7	97.6	167.1	313.5	380.4	441.7
19	96.4	114.7	191.9	387.3	475.9	556.6
22	108.4	128.4	213.0	457.3	568.4	668.2
25	120.1	143.3	234.2	518.8	663.0	779.3
28	131.2	157.7	256.8	578.2	757.4	890.1
30	138.6	166.2	270.9	615.0	818.9	961.1

added in the form of bacto-yeast-extract, and in the same basic medium without nitrogen. The total amounts of oxygen consumed by the organisms in the different media are shown in table 1. From the data in the table the rates of oxygen consumption were calculated. These are shown graphically in figure 1.

The results show that the total amount of oxygen consumed varied with the amount of nitrogen in the medium. It is evident, however, that respiration was being carried on by the organism in the no-nitrogen medium, but the amount of oxygen consumed was not large, when compared with the amount consumed in media containing appreciable amounts of nitrogen. Furthermore, the oxygen consumption decreased at nearly a uniform rate throughout the

experiment. This would indicate that although the cells were alive and respiring, they were not multiplying, and furthermore, that their respiration rate was gradually decreasing. In other words, they might be looked upon as resting cells, as the term is used by Quastel and his associates (14).

In the various media containing nitrogen the organisms reacted somewhat differently. The nitrogen stimulated growth and multiplication of the cells in the cultures, resulting in an increased rate of oxygen consumption, which in turn led to the consumption of larger amounts of oxygen.

In the medium with only 0.72 p.p.m. of nitrogen the stimulation was not great but it was definite between the fourth and eighth hours. After the eighth hour the rate decreased for a few hours, after which it was fairly uniform, the rate curve being almost parallel with the rate curve of the no-nitrogen medium during the same period. This indicates that during the early part of

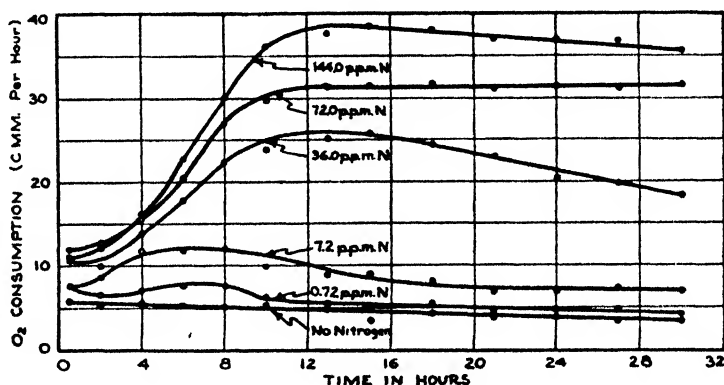


FIG. 1. THE RATE OF OXYGEN CONSUMPTION BY *Rhizobium leguminosarum* IN YEAST EXTRACT MEDIA

the experiment growth was stimulated by the nitrogen. The amount of nitrogen available, however, was extremely small and was soon used by the organisms. After it was all used the rate of oxygen consumed decreased and then continued at a low uniform rate. It appears that after utilization of the available nitrogen the cells continued to respire but did not grow and were in a state similar to the resting cells in the no-nitrogen medium.

Where larger amounts of nitrogen were available practically the same influence on the rate of oxygen consumption occurred, except it was magnified almost in proportion to the increase in nitrogen content in the medium. The increase in rate of oxygen consumption was very rapid and continued for a period of about 12 hours. During this period the organisms were undoubtedly growing rapidly and it is quite probable that they were in their logarithmic phase of growth. After that time the rate in most cases was fairly uniform, except for a slight decrease in the medium with 36.0 p.p.m. of nitrogen, indicating that the organisms were probably in a resting condition. These findings

TABLE 2

*Oxygen consumption by Rhizobium leguminosarum in media containing ammonium chloride*

TIME	OXYGEN CONSUMPTION BY CULTURE					
	No nitrogen	Amounts of nitrogen as ammonium chloride in media				
		0.72 p.p.m.	7.2 p.p.m.	36.0 p.p.m.	72.0 p.p.m.	144.0 p.p.m.
hrs.	c.mm.	c.mm.	c.mm.	c.mm.	c.mm.	c.mm.
0	0.0	0.0	0.0	0.0	0.0	0.0
2	10.5	11.6	7.4	7.8	8.8	10.2
4	21.2	23.8	16.2	16.1	17.0	17.5
6	32.9	33.5	23.7	22.3	23.4	22.9
8	42.7	43.6	31.1	30.5	30.4	28.7
10	53.8	55.5	39.6	39.7	40.2	36.2
12	62.4	65.9	47.1	46.8	47.9	43.7
15	76.5	82.8	61.4	58.7	61.0	56.7
18	91.7	99.3	75.3	74.3	75.7	69.6
20	100.2	110.4	82.9	83.1	84.4	76.7
22	109.5	125.3	92.1	92.6	93.9	84.4
24	118.1	141.7	103.0	105.3	104.4	92.6
26	127.4	160.3	117.1	124.7	115.6	102.4
28	135.8	179.5	132.7	139.1	130.0	111.2
30	144.4	198.4	147.6	158.9	144.0	118.7

TABLE 3

*Oxygen consumption by Rhizobium leguminosarum in media containing sodium nitrate*

TIME	OXYGEN CONSUMPTION BY CULTURE					
	No nitrogen	Amounts of nitrogen as sodium nitrate in media				
		0.72 p.p.m.	7.2 p.p.m.	36.0 p.p.m.	72.0 p.p.m.	144.0 p.p.m.
hrs.	c.mm.	c.mm.	c.mm.	c.mm.	c.mm.	c.mm.
0	0.0	0.0	0.0	0.0	0.0	0.0
2	17.0	16.8	14.6	14.6	15.8	15.6
4	27.1	27.2	25.2	23.8	25.2	23.1
6	37.7	39.1	35.1	33.4	34.8	30.3
8	47.3	48.2	44.1	42.3	43.5	37.0
10	56.2	58.0	54.4	49.8	51.7	45.2
12	66.9	68.6	66.4	59.6	61.6	56.5
14	77.0	79.3	77.7	69.1	71.0	67.4
16	86.4	90.2	91.0	78.7	81.5	79.4
18	94.7	101.7	100.8	86.8	89.9	89.9
20	103.2	114.6	113.1	94.9	99.7	100.6
22	...	129.9	126.9	107.3	111.4	113.6
24	125.5	145.7	140.7	117.9	123.1	126.2
26	134.3	161.5	152.9	128.4	135.1	139.2
28	141.5	175.5	165.1	139.4	147.1	152.2
30	149.9	191.3	179.9	151.8	159.9	166.7

TABLE 4

*Oxygen consumption by Rhizobium leguminosarum in media containing urea*

TIME	OXYGEN CONSUMPTION BY CULTURE					
	No nitrogen	Amounts of nitrogen as urea in media				
		0.72 p.p.m.	7.2 p.p.m.	36.0 p.p.m.	72.0 p.p.m.	144.0 p.p.m.
hrs.	c.mm.	c.mm.	c.mm.	c.mm.	c.mm.	c.mm.
0	0 0	0 0	0 0	0 0	0 0	0 0
2	10.2	8.9	7.8	7.4	6.3	7.7
4	16.6	14.6	14.6	13.5	12.6	13.1
6	22.8	20.1	23.6	18.7	18.2	18.5
8	29.0	25.3	27.6	23.2	23.4	23.5
10	35.7	32.3	32.5	28.3	28.1	28.5
12	42.2	39.4	37.7	34.7	34.6	35.2
15	53.3	49.1	48.2	42.9	42.7	42.7
18	60.5	59.2	57.7	52.8	51.4	50.2
21	70.9	70.2	68.6	63.3	61.4	58.6
23	76.3	76.3	74.3	68.4	65.3	61.7
25	82.1	82.5	80.7	75.3	71.4	65.4
28	90.7	92.2	90.6	85.2	79.5	70.8
30	96.3	98.4	94.4	91.3	84.4	73.8

TABLE 5

*Oxygen consumption by Rhizobium leguminosarum in media containing alanine*

TIME	OXYGEN CONSUMPTION BY CULTURE					
	No nitrogen	Amounts of nitrogen as alanine in media				
		0.72 p.p.m.	7.2 p.p.m.	36.0 p.p.m.	72.0 p.p.m.	144.0 p.p.m.
hrs.	c.mm.	c.mm.	c.mm.	c.mm.	c.mm.	c.mm.
0	0.0	0.0	0.0	0.0	0.0	0.0
2	13.3	7.7	14.4	10.7	14.0	21.7
4	22.4	16.6	20.1	15.9	20.3	33.1
6	31.0	23.3	23.6	24.7	31.7	48.7
8	39.6	30.7	30.0	31.8	39.4	60.9
11	50.7	42.6	40.3	43.1	52.7	77.2
14	61.8	53.8	53.6	52.6	62.1	90.1
17	72.3	65.7	71.7	62.9	73.4	103.7
20	81.6	77.6	80.2	72.8	84.2	117.6
23	92.1	91.0	97.9	82.0	94.0	130.2
26	103.5	106.3	127.9	93.7	106.6	145.7
28	111.3	116.3	144.9	101.8	114.3	156.0
30	118.1	123.0	156.2	106.0	119.9	164.8

are in accord with the earlier conclusions of Walker, Anderson, and Brown (16) concerning the period of the logarithmic phase of growth for *Rhizobium*.

Tables 2, 3, 4, and 5 show the results obtained in similar oxygen consumption experiments where other forms of nitrogen, ammonium chloride, sodium nitrate, urea, and alanine, were used in place of the yeast extract. The rates

of oxygen consumption were calculated from these data and some representative results are shown in figure 2.

These data indicate that *Rhizobium leguminosarum* reacted entirely differently when nitrogen was supplied in forms other than yeast extract. In general, the rate of respiration, and therefore, the total amount of oxygen con-

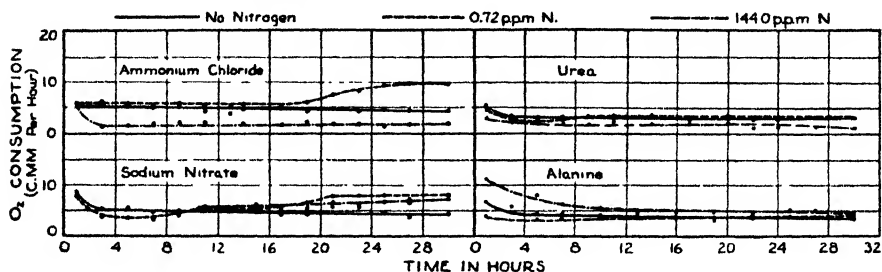


FIG. 2. THE RATE OF OXYGEN CONSUMPTION BY *Rhizobium leguminosarum* IN MEDIA CONTAINING AMMONIUM CHLORIDE, SODIUM NITRATE, UREA, AND ALANINE

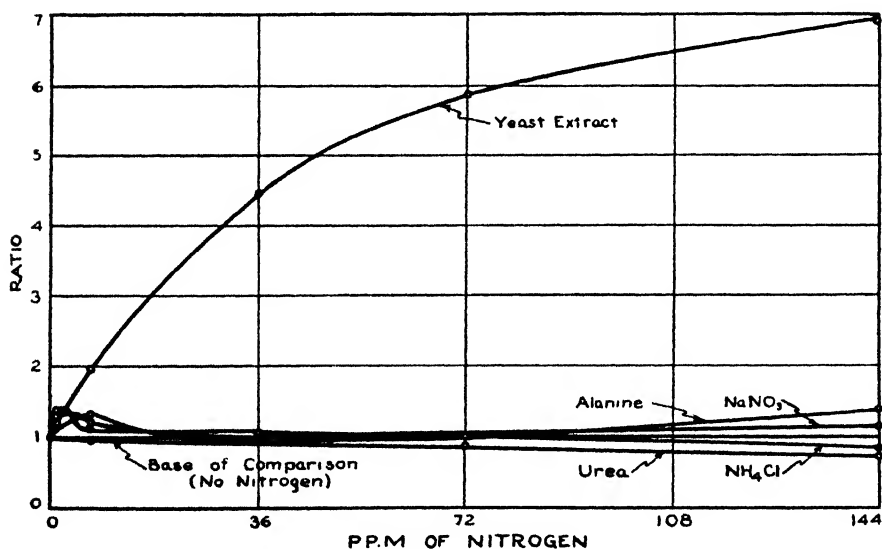


FIG. 3. RATIO OF THE AMOUNT OF OXYGEN CONSUMED IN 30 HOURS IN MEDIA CONTAINING VARIOUS NITROGEN SOURCES TO THAT CONSUMED IN A NITROGEN-FREE MEDIUM

sumed, was very nearly the same in all of the media, irrespective of the source or concentration of nitrogen used. In no case was there any large stimulation of oxygen consumption in the media containing nitrogen. The larger amounts of urea and ammonium chloride, however, depressed oxygen consumption slightly, but no depression of oxygen consumption occurred with the sodium nitrate or the alanine.

Figure 3 shows graphically the ratio of the total amounts of oxygen con-

sumed in the different concentrations of the various nitrogenous materials compared with that consumed in the no-nitrogen medium at the end of 30 hours. These curves show definitely the difference in the response of the organisms to the various materials. The yeast extract was decidedly stimulative in all concentrations, whereas the other materials appeared to be only slightly stimulative in the lower concentrations, and of little or no effect in the medium concentrations. In the higher concentrations the alanine and sodium nitrate were slightly stimulative, and urea and ammonium chloride were slightly depressive. The extent of the variations from the no-nitrogen medium induced by the nitrogenous materials other than the yeast extract is so small in all cases that they are of doubtful significance.

#### DISCUSSION

The data as a whole indicate that the yeast extract definitely stimulated the growth of *Rhizobium leguminosarum*, whereas the other nitrogenous materials used did not appreciably stimulate growth. The organisms in the media containing ammonium chloride, sodium nitrate, urea, or alanine reacted as they did in the medium with free nitrogen as the sole source of nitrogen. In these media the organisms were, presumably, respiring but they were in a resting state and were not growing.

The results naturally bring up the question of the suitability of these various forms of nitrogen for the growth of the organisms. It is certain that the yeast extract stimulated growth, but whether it was the nitrogen of the yeast extract that was effective remains to be demonstrated. The yeast extract used has a nitrogen content of 7.6 per cent. Sadler, Eagles, and Pendray (15) reported the following for the nitrogen content of bacto-yeast-extract, which is similar to that used in these experiments:

	per cent
Protein nitrogen.....	0.0
Proteose nitrogen.....	0.0
Peptone nitrogen.....	24.2
Sub-peptone nitrogen.....	75.8

It is evident that the nitrogen of the yeast extract exists in the simpler degradation products of protein decomposition, or of the simplest combinations of the amino acids, and presumably nitrogen in this form is suitable for the growth of *Rhizobium*. Then there is also the possibility that in the presence of the other constituents of the yeast extract the organisms were better able to utilize the free nitrogen of the atmosphere.

It is entirely possible that there were certain constituents in the yeast extract other than the nitrogen that caused the stimulation in oxygen consumption. It is not known what these may have been, but undoubtedly certain readily available carbonaceous materials were present. It is a question, though, whether such materials could have been much more effective in stimulating oxygen consumption than the glucose, which was present in all the media.

Glucose ordinarily offers a readily available source of energy for the organisms and is usually considered entirely satisfactory for this purpose. The use of mannitol in yeast extract medium has been recommended by Fred and Waksman (9), and is used extensively by some investigators working with *Rhizobium*. In comparative tests, however, respiration experiments with mannitol in the medium in place of glucose have shown no superiority for the mannitol in stimulating growth, as determined by oxygen consumption.

There is also the possibility that the yeast extract contained some accessory food constituent such as vitamins or auximones. Bottomley (4) and Mockridge (12) concluded that certain microorganisms synthesize accessory food constituents, which they called "auximones." When materials containing auximones were supplied to growing plants along with a complete nutrient solution there was a direct stimulation of the growth of the plants due to the auximones. The beneficial effects of bacterized peat were attributed to the auximones produced by the bacteria. If it is possible for microorganisms, and particularly yeasts in this case, to produce such constituents, then the stimulation of the growth of *Rhizobium* may have been due to the auximones of the yeast extract.

Then too, if yeast cells are capable of synthesizing vitamins, and if vitamins stimulate the growth of *Rhizobium*, then it is possible that the stimulation may have been due to the vitamin content of the yeast extract. Werkman (18), from a study of the effects of vitamins on the growth of *Rhizobium* and *Azotobacter*, came to the conclusion that the stimulation was due to the additional amount of energy material added to the cultures and not to the vitamin content of the material added. His work would indicate that the stimulation of *Rhizobium* by the yeast extract was due to some factor other than auximones or vitamins.

Furthermore, it is entirely possible that some mineral constituent of the yeast extract was the stimulative agent. The effects on the growth of *Rhizobium* of small amounts of such elements as manganese, boron, and others that in the past have not been considered essential for plant growth, have not been investigated.

It is clearly evident that something other than the nitrogen content of the yeast extract may have stimulated the growth of *Rhizobium* in these experiments. Further work has been planned and is now under way to study the factors controlling this stimulation, with special emphasis upon the effects of the nitrogen factor alone. The results of this work will be presented in later publications.

#### SUMMARY AND CONCLUSIONS

Experiments have been conducted to study the effects of nitrogen of different sources and in various concentrations upon the growth of *Rhizobium leguminosarum*. The effects were interpreted from changes in the rate of oxygen consumption as measured in respiration chambers connected with micromanometers of the type used by Warburg.



In the media without nitrogen the organisms continued to respire at a low uniform rate throughout the various tests. Presumably they were in the resting state, and no growth occurred in the cultures.

In a similar medium except where nitrogen was added in the form of bacto-yeast-extract, the growth of *Rhizobium* was stimulated almost in direct proportion to the amount of the extract added. The rate of oxygen consumption increased during the first 12 hours, after which it remained constant or decreased slightly. Presumably after the organisms had utilized all of the nitrogen in the culture, or after they had passed the logarithmic phase of growth they were in the resting state, and no growth occurred.

In media containing nitrogen added in the form of ammonium chloride, sodium nitrate, urea, or alanine, there was no marked stimulation of growth irrespective of the concentration of nitrogen in the medium, and presumably the cells remained in the resting state throughout the experiment. There is some evidence that oxygen consumption by the organisms was depressed by ammonium chloride and also by the urea in the higher concentrations employed.

Whether the stimulation of growth induced by the yeast extract was due to its nitrogen content or to some other constituent of the extract is not known. It is entirely possible that the particular form or combination of nitrogen in the yeast extract is especially suitable for use by *Rhizobium*. It is also possible, however, that the stimulation of growth was induced by energy furnishing material, accessory food constituents, or certain elements necessary for the growth of the organisms which were not supplied in the basic medium.

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## PLATE 1

## APPARATUS FOR MEASURING THE EFFECTS OF NITROGEN ON RHIZOBIUM

FIG. 1. Respiration vessel. The culture of bacteria may be seen in the bottom of the vessel and the KOH in the inside tube.

FIG. 2. Simple manometer of the Warburg type with respiration vessel attached.

FIG. 3. Water bath in which the temperature of the water surrounding the respiration vessels was thermostatically controlled within  $0.01^{\circ}\text{C}$ .

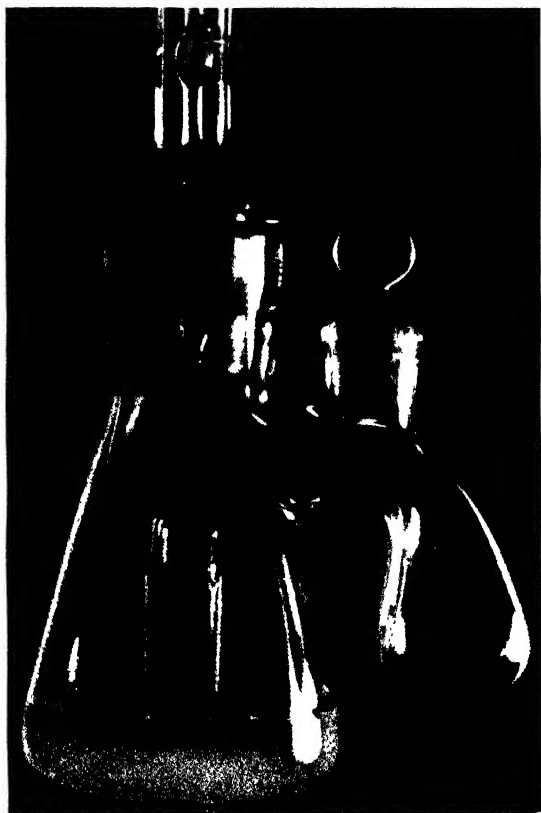


Fig. 1

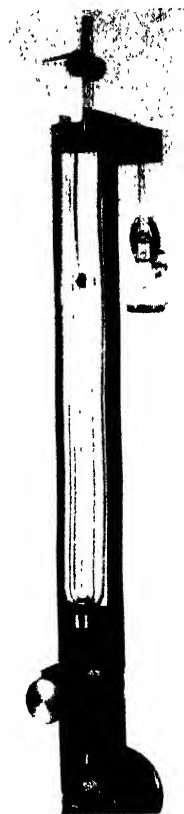
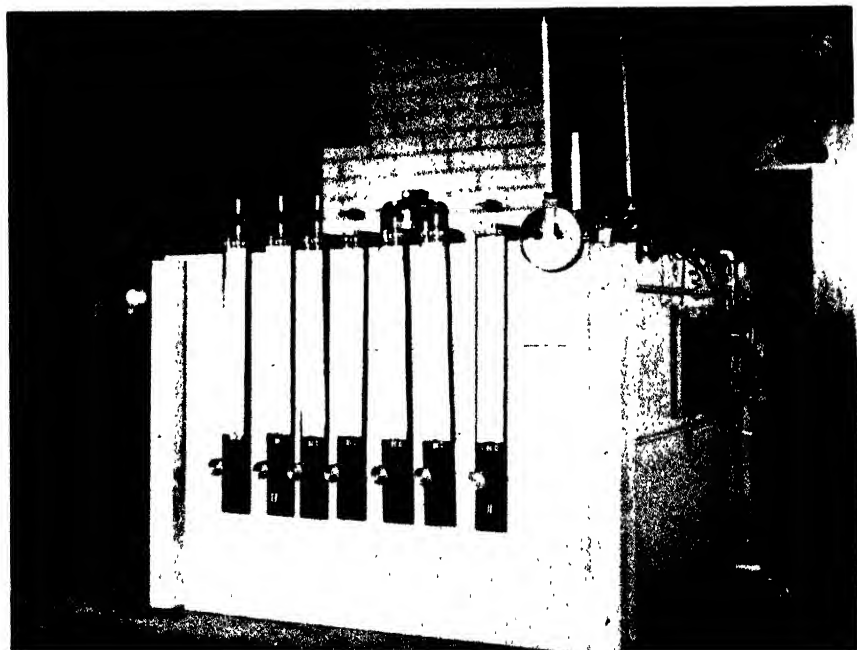


Fig. 2





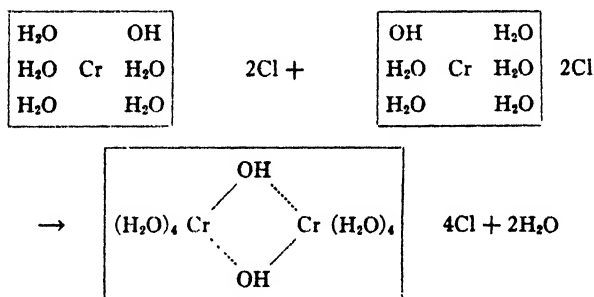
# LAWS OF SOIL COLLOIDAL BEHAVIOR: XIV. AGING OF COLLOIDS AND BASE EXCHANGE<sup>1</sup>

A. J. PUGH<sup>2</sup>

*New Jersey Agricultural Experiment Station*

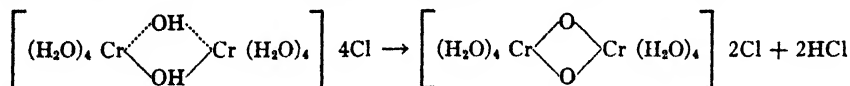
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The reactions shown by many complicated materials such as the soil, silicates, and complex salts, are very difficult to represent by any simple dissociation formulas. Thomas and Whitehead (16) obtained results with aluminum oxychloride, which could be interpreted on the Werner theory, but for which the ordinary dissociation equations were inadequate to explain the observed facts. A similar explanation had previously been advanced by Bjerrum (1) to account for the increase in molecular weight of basic chromic sulfate on aging. Their explanation for both the chromic and the aluminum salt is identical, and the change that takes place is represented as follows:



the two hydroxyl groups being combined through residual valencies to give a larger molecule. This process, termed "olation," accounts for the increase in molecular weight and for the diminished activity of the hydroxyl groups.

Stiasny (15), in order to interpret the increased acidity and the decreased solubility of chromic salt on aging, made the second assumption that the hydrogen wandered from the oxygen, giving rise to "oxolation," with the secondary formation of a true valence bond between the oxygen atoms and the metallic element:



<sup>1</sup> Journal Series paper of the New Jersey Agricultural Experiment Station, department soil chemistry and bacteriology.

The work reported in this paper was done in conjunction with the researches of Sante Mattson, to whom the author is indebted for guidance and encouragement. It is for this reason that the paper appears in Mattson's series "Laws of Soil Colloidal Behavior."

<sup>2</sup> Formerly British Ministry of Agriculture Research Scholar, stationed at Rothamsted 1928-1931.

The object of this investigation was to extend this work and to apply the results, so obtained, to the investigation of base exchange. The effect of aging was studied with both aluminum hydroxide and with aluminum silicate, and a different theory based on atomic structure is advanced to account for the results, which substantiate the previous investigations of other workers.

#### PREPARATION OF ALUMINUM HYDROXIDE SOL

Ten grams of aluminum hexahydrate was dissolved in CO<sub>2</sub>-free water, and aluminum hydroxide was precipitated with sodium hydroxide until flocculation commenced, the hydrogen-ion concentration of the supernatant liquid being adjusted to the isoelectric pH 8.2. The sol was centrifuged, the supernatant liquid decanted, CO<sub>2</sub>-free water added, and the process repeated until a little peptization was observed. The floc was subsequently transferred with distilled water to a flask and protected from CO<sub>2</sub>. Twenty cubic centimeters of the suspension was placed in boiling tubes, and the pH determined in each one at intervals with the quinhydrone electrode, at a temperature of 25°C.

TABLE 1

*Effect of aging on pH of aluminum hydroxide*

	TIME IN DAYS												
	0	1	2	3	4	5	6	7	8	9	10	11	12
pH	7.31	7.11	6.35	6.15	5.90	5.90	5.67	..	5.65	5.60	5.61	5.33	5.33
													4.67

Equilibrium was usually reached in 30 minutes. The results are given in table 1.

The increase in hydrogen-ion activity with time is surprising, and at the end of 40 days the pH was only 4.67. Although the interpretation on Werner's theory is sufficient to explain the results, the original idea of Werner has been superseded by the development of theories of valency based on atomic structure, and for the interpretation of results obtained with silicates, the theory is much more advantageous. A brief review of the modern conception of atomic structure, all that is required to discuss the results obtained, is given below, but for greater detail the reader is referred to Sidgwick (4).

#### ELECTRONIC INTERPRETATION OF VALENCY

Prior to 1913, there existed no very definite relation between structure and valency, but at this time atomic numbers were discovered. This gave the number of electrons in each atom and indicated that the numbers in the inert gases were capable of a very stable arrangement. Kossel (6) pointed out that the element before an inert gas is a strongly electronegative halogen, and immediately following occurs a strongly electropositive alkali metal. These ele-

ments would then combine because of their tendency to reach the stable arrangement of an inert gas. Thus in the series

S	Cl	A	K	Ca
16	17	18	19	20

eighteen electrons must be capable of a very inert and stable arrangement since argon contains this number. If potassium would lose one electron to the chlorine, both would achieve this stable number, and calcium could do so by giving 2 electrons to 2 chlorine atoms. This would give, on dissociation of the compound, the stable ions Cl, K, Ca, all with 18 electrons, the chlorine ion with one more than necessary to balance the charge in its nucleus, the potassium with one less than necessary to balance the charge on its nucleus. On this theory the valency of an atom is the number of electrons it must gain or lose in order to reach the stable number of an inert gas, but the elements would have to be utterly dissimilar in character.

There are molecules in which it is impossible to distinguish between the positive and negative atom, as when two hydrogen atoms combine to form a molecule, and on Kossel's theory such a combination could not be accounted for. The solution to the difficulty was given by Lewis (8), who, like Kossel, assumes that the cause of chemical combination is the tendency of electrons to distribute themselves so as to reach the stable arrangement of the inert gases, but he made a further assumption of great importance, that is, that atoms can also share electrons so that the shared electrons contribute in some way to the stability of both atoms. Chlorine (17)<sup>3</sup> has one electron less than the stable number of argon (18). Hence, it readily takes up one from an atom like potassium (19) to form potassium chloride, a compound which readily ionizes in solution and is also ionized even in the solid state. It can also complete its number of electrons by combining with another chlorine atom, if it shares one electron belonging to another chlorine atom; and if at the same time the second chlorine atom shares an electron belonging originally to the first, then each is satisfied. The Lewis method permits a smaller number of electrons to do the work of what on the Kossel model would require more. To distinguish these two forms of linkage Langmuir suggested the terms "electro-valency" and "co-valency," the former giving the linkage in potassium chloride, the latter in molecules such as molecular chlorine or hydrogen.

The necessity of two shared electrons to every co-valent link forms the basis of Lewis's theory, the process being the same as the linking of hooks, so that the molecule is, as it must be, electrically neutral. The single link between potassium and chlorine, or between two chlorine atoms, both satisfies the single valency of potassium and of chlorine, in both cases, and gives the stable member of an inert gas. Thus the theory of Lewis provides a mechanism for two kinds of linkage, "polar" and "non-polar," which the chemical evidence requires.

<sup>3</sup> The number in parentheses immediately following the name of the element refers to its atomic number on electronic arrangement.



No atomic model is involved in the theories, but subsequently Bohr advanced a theory that electrons are distributed in shells around the proton nucleus, each shell corresponding to a definite energy level. In hydrogen the kernel of the atom is the nucleus itself, and it has one valence electron. The two electrons of helium are not regarded as valence electrons, and it is considered that the whole of the atom represents the kernel. The pair of electrons in helium constitute the shell, and a similar pair will appear as the first shell of all atoms of higher atomic numbers. The atomic number and kernel of the atoms required for this discussion are given in table 2.

The electrons in the outer shell are the valence electrons. Thus hydrogen can share its electron to reach the stable number of helium by combining with

TABLE 2  
*Atomic number and kernel of various atoms*

ELEMENT	ATOMIC NUMBER	KERNEL OF ATOM GROUPS			
		1	2	3	4
H	1	1			
He	2	2			
Li	3	2 + 1			
Be	4	2 + 2			
B	5	2 + 3			
C	6	2 + 4			
N	7	2 + 5			
O	8	2 + 6			
F	9	2 + 7			
Ne	10	2 + 8			
Na	11	2 + 8 + 1			
Mg	12	2 + 8 + 2			
Al	13	2 + 8 + 3			
Si	14	2 + 8 + 4			
A	18	2 + 8 + 8			
Fe	26	2 + 8 + 13 + 3			

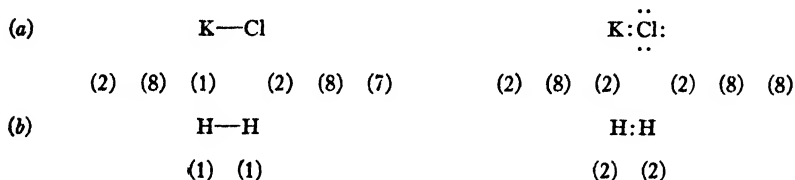
chlorine (2) (8) (7), whereas lithium combines with chlorine if it loses one electron to reach the stable number of helium. Sodium can lose its valence electron if it combines with chlorine, the sodium achieving the number in the next gas neon (2) (8), the chlorine by gaining one reaching the number in argon (2) (8) (8). Helium with only a pair of electrons has the same stability as the remaining rare gases with an outer shell of eight, the octet. It was therefore originally supposed, that the redistribution of electrons on chemical combination led to the production of an outer group of eight, with all atoms except the immediate neighbors of helium, where the number is two. This was the foundation of the octet theory of Lewis, on a cubic model of the atom. Although the "static atom" has been abandoned, it is still true that the stability of a large number of compounds is determined by their having in combination a group of eight shared electrons, and since a pair of electrons constitutes a single bond, a co-valency of four.

On this basis a mechanism is given for two kinds of linkage:

(a) Polar or ionized linkage, as in potassium chloride, due to the transference of an electron from one atom to the other.

(b) Non-polar, non-ionized linkage (co-valency), due to the sharing of electrons two to each link as in the combination of atoms to form molecules.

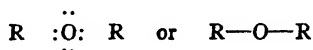
Both are represented by two dots, to represent a single bond:



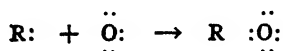
And in combination the shared electrons contribute to the stability of both atoms, reaching the inert gas number.

It should be noted that hydrogen achieves stability with only two shared electrons. The chlorine in potassium chloride achieves stability by completing the octet or its outer shell; the outer shell of the potassium however consists of only two shared electrons. In order to maintain the octet theory, it has been suggested that the inner shell of the potassium be regarded as the octet. It is unnecessary however as such compounds are never really combined, the group (2) (8) (2) being almost always a highly ionized compound, such as the strong electrolytes.

There is another mode of combination of very great importance and first suggested by Lewis. A divalent electronegative atom like oxygen (2) (6) achieves stability on combination by gaining electrons to reach the stable number of neon (2) (8). It can gain these by forming two links, that is, by sharing two electrons belonging to another atom or atoms while the other atom shares two belonging to the oxygen:



This is the usual divalent oxygen of structural chemistry. The new mode of combination suggested by Lewis is that oxygen forms only a single co-valency, shares only two electrons with the other atoms, but that both of these come from the other atoms:

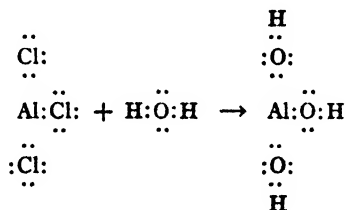


This constitutes only a single link, but it increases the valency electrons of the oxygen by two, and it becomes saturated, achieving the number of the inert gas neon. Lewis' suggestion is that two shared electrons which constitute a co-valency can come in two ways: (a) as they usually do, by each of the atoms contributing one of them; or (b) by one of the atoms contributing both. The

latter is termed a "co-ordinate link" or "semi-polar bond," and gives an explanation of the co-ordination compounds of Werner. It gives peculiar properties to a molecule, and in particular a very strong external electrostatic field, but it is much more easily broken than an ordinary co-valency, and the compound can again be broken up into simple molecules. This is the type of linkage that apparently occurs in aluminum hydroxide, and probably in the clay fraction of the soil, both aluminum hydroxide and clay being susceptible to hydrolysis.

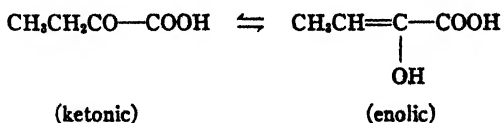
#### AGING OF ALUMINUM HYDROXIDE SOL

The formation of a hydroxide from a chloride such as aluminum chloride is a hydrolysis, the preliminary reaction being the dissociation of chlorine, which is combined with aluminum by electro-valencies, and the compound being highly ionized:

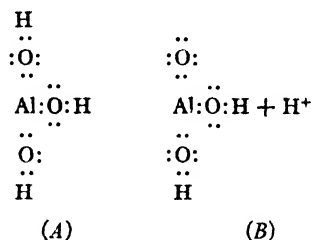


The  $\text{Cl}^-$  on dissociating takes away the original electrons of the aluminum (2) (8) (3), giving the  $\text{Cl}^-$  ions. The aluminum subsequently combines with the  $\text{OH}^-$  ion, but both the electrons come from the negative oxygen, whether from water or alkali. This is a co-ordinate co-valency, or a semi-polar link, on the theory as previously outlined, as both electrons necessary for the formation of a single link are obtained from one atom, the oxygen, of the hydroxyl ion. The aluminum is unsaturated, however, as it has not during the formation of the hydroxide achieved the stable number of the inert gas, that is, its outer group has only six shared electrons and not the eight of the inert gases. This phenomenon of atomic unsaturation is the electronic interpretation of Werner's residual valency.

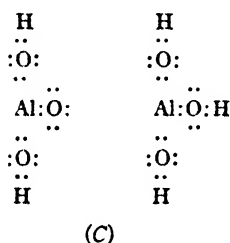
Suspending the aluminum hydroxide in water leads to a rearrangement which can be easily interpreted on this theory. The hydrogen ion is the smallest ion known, and because of this it is particularly free from the "steric" effects to which other larger ions are susceptible, as in the phenomenon of tautomerism that occurs in aceto-acetic ester, where the hydrogen wanders between the carbon and the oxygen, giving rise to two compounds of the same molecular weight:



The first step that occurs is the wandering of hydrogen from a molecule (*A*) of the aluminum hydroxide to give a molecule (*B*):



The oxygen will retain the pair of electrons, but a rearrangement will take place, by a molecule such as (*B*) combining with another molecule such as (*A*), the aluminum of molecule (*A*) achieving stability by combining with the lone pair of electrons of the oxygen of molecule (*B*), the aluminum of (*A*) thus becoming saturated, by a co-ordinate co-valency giving molecule (*C*).



The molecular weight thus consistently increases and the process is capable of an indefinite formation, the molecule always remaining unsaturated, or it may possibly go through a rearrangement to give a complicated ring structure after some time. Since the pH of the suspension apparently diminishes consistently with time, the latter is improbable. The formations of the bonds always arise through a single linked oxygen —O— and not O=, and would be difficult to represent on any theory other than on that based on atomic structure, which gives a reasonable explanation of both the increase in molecular weight and the increased acidity of the suspension, the hydron combining with the remaining salt, sodium chloride, giving hydrochloric acid, and, because of the internal rearrangement of the molecules of hydroxide itself, there is no place for it again to re-enter the molecule, the electrons of the oxygen attaching themselves to the unsaturated aluminum.

#### AGING OF ALUMINUM SILICATE AND ADSORPTION OF BARIUM BY THE COLLOID ON AGING

Although the interpretation of the data for aluminum hydroxide is comparatively easy, the chemistry of silicates, which is of the utmost importance for soil workers, is much more complicated. In order to study the phenomena of aging with aluminum silicate, the compound was prepared from the chloride

by mixing aluminum chloride and sodium silicate and adding sodium hydroxide until flocculation commenced. The floc was centrifuged and washed with  $\text{CO}_2$ -free water until a slight peptization was observed, as with the hydroxide, and kept in distilled water in a bottle protected from  $\text{CO}_2$ . The pH of the suspension was determined at weekly intervals with a quinhydrone electrode, and simultaneously 50 cc. of the sol was suspended in 100 cc. of barium acetate, the total volume of the sol and barium acetate being made up to 200 cc. for a study of the barium adsorption and flocculation. After a time the sols were centrifuged, and the barium in the supernatant liquid was determined. The difference between the original barium content of the barium acetate and that in the supernatant liquid was taken as being the quantity of adsorbed barium. The results are given in table 3.

The sol thus shows the same effect of aging that was evident with aluminum hydroxide, the pH has a tendency to decrease and shows a considerable diminution the first week. This phenomenon will be discussed first, and the second effect of diminished adsorption on aging will be discussed later.

TABLE 3  
*Effect of aging on the pH, and adsorption capacity of aluminum silicate*  
50 cc. sol = 3.02 gm.

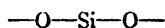
AGE	pH OF SOL	Ba <sup>++</sup> ADDED	Ba <sup>++</sup> IN SUPERNATANT LIQUID	Ba <sup>++</sup> ADSORBED	Ba <sup>++</sup> ADSORBED BY FRESH SOL
<i>days</i>		<i>m e</i>	<i>m e</i>	<i>m. e.</i>	<i>m e.</i>
0	4 61	11 055	9 408	1 647	100
7	4 52	11 055	9 824	1 231	74 7
14	4 52	11 055	9 976	1 079	65.5
21	4 52	11.055	10 088	0 967	58 7

The normal state of combination in a molecule is reached when each atom of hydrogen has its stable pair of electrons, as in helium, and each other atom the stable group of eight, with the exception of the highly dissociated compounds such as potassium chloride, where even then the inner group can be taken as the octet. A combination of (2) (8) (2) is, however, always highly ionized. Molecules are achieved by atoms sharing between them a pair of electrons that constitute a single bond. In a double bond formation this, however, cannot occur, as in the combination of atomic oxygen to give the molecule. Oxygen (2) (6) has six valence electrons and requires a share in two more in order to complete its octet. Even if two atoms share an electron pair, this does not complete the octet of the two atoms. If, however, two electron pairs are shared, then each atom can be said to have its outer group of eight  $\text{:}\ddot{\text{O}}\text{:}\ddot{\text{O}}\text{:}$ , the four shared electrons now counting independently toward the stability of both atoms and giving the double bond  $\text{O} = \text{O}$ .

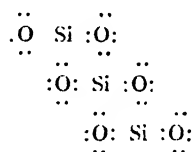
Double bonds, such as those for oxygen, occur often with carbon compounds.

A consideration of the great difference in physical properties between the compounds of carbon and of silicon, which have the same valency [C (2) (4), and Si (2) (8) (4)], led Lewis (8) to suggest that multiple bonds were confined to the elements of the first period of eight, and especially to carbon, nitrogen, and oxygen. Carbon dioxide and silicon dioxide are totally unlike in physical properties, and the two formulas,  $O = C = O$  and  $O = Si = O$ , in no way account for the great dissimilarity in the physical properties of the two substances.

If silicon is incapable of forming double bonds, then in terms of the older valence theory, silicon dioxide would have to be represented as

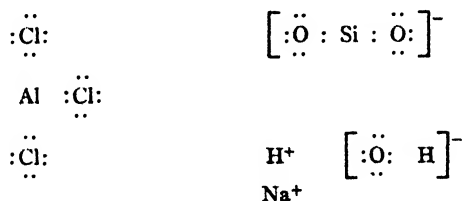


and the uncompleted bonds could be completed only by union with other molecules. In terms of the newer theory, it would be represented as follows, a pair of electrons forming a single link  $: \ddot{O} : Si : \ddot{O} :$ . With such a structure the oxygen has a lone pair, and the silicon only four, instead of the eight required for stability. The molecules would combine to form larger molecules as of the following type:



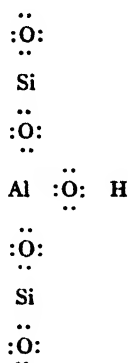
In such a formula the existence of unsatisfied valences is indicated, and the process of polymerization would go on indefinitely until all the material was exhausted, as with the aging of aluminum hydroxide sol.

When sodium silicate is added to aluminum chloride, the following ions are in the solution:

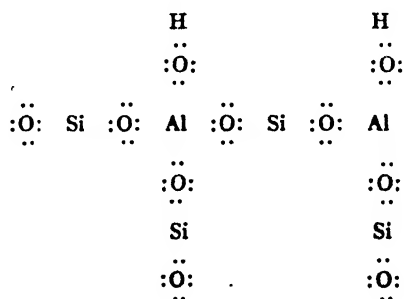


and as the electrovalent chlorine dissociates, the silica or the hydroxyl ion will become attached to the aluminum through the lone pair of the oxygen, the two electrons necessary for the formation of a bond being given by one and the same atom, thus constituting a co-valency. The following formula is given as

an illustration of the type of bond, but the two oxygen atoms of the silica may be giving their lone pairs of electrons to the aluminum:



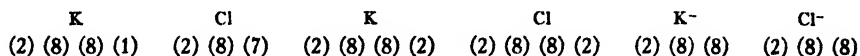
A compound of this structure is highly unsaturated, the aluminum having only six shared electrons and the silicon only four. In order to reach the stable group of eight, the molecules will polymerize, union taking place between the molecules either at the silicon or aluminum atoms, through the oxygen of the silicon fraction or through the oxygen of the hydroxide group, after the H ion dissociates, giving a compound of the following type:



which is capable again of indefinite polymerization until all the material is exhausted. The important point, however, is that when the chlorine dissociates from the aluminum chloride, it takes the bonding pair of electrons with it, and for the formation of the silicate or the hydroxide, the bonding pair are contributed by the lone pair of the oxygen of the silica or hydroxyl ions. This is by definition a co-valency (co-ordinate), both electrons coming from one and the same atom.

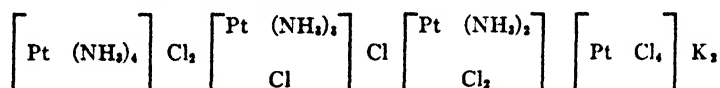
A clear distinction must be drawn between a co-valency and an electro-valency, the normal co-valence that exists between like atoms to form molecules being neglected. Why there should be so great differences in properties of compounds, one with an electro-valence and the other a co-valence, cannot adequately be answered. That they are two different forms of linkage is, however, upheld by Sidgwick (14). In an ionized compound, such as potassium

chloride, which results from the transference of an electron from the potassium to the chlorine, thus,

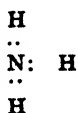


with subsequent dissociation, a true bond, it is believed is never formed, the ions being held together purely by electrostatic attraction, and even in the solid state the potassium chloride is ionized. In co-valent compounds, however, the links form part of the constitution of each atom in the molecule, and co-valencies, unlike electro-valencies, are directed forces, and beyond limits of slight distortion their position in space, like those of carbon compounds, is fixed, and it is quite possible to have the same groups arranged around the same atom in more ways than one, giving rise to structural isomerism. The conditions of when a co-valency changes into an electro-valency will be discussed when we come to base exchange.

The distinction was first drawn by Werner, with compounds formed by metallic salts. He showed that in the platinum amines, the platinum is at the center of a co-ordination zone, in which a number of atoms are attached to the central atom, and this number he called the co-ordination number. Those atoms within the co-ordination shell, represented by brackets, were supposed to be bound by true valences and were ionized linkages. Thus in the following series of compounds with platinum:



as the  $\text{NH}_3$  is replaced by  $\text{Cl}$ , the conductivity of the compounds diminishes. And the third compound was found by Werner to be a non-electrolyte. On further substitution of the  $\text{NH}_3$  by  $\text{Cl}$ , the charge of the compound changes from the original of a double positively charged particle to a double negatively charged particle. All the ions or radicals that can enter a co-ordination group have a lone pair of electrons as the chlor ion  $:\ddot{\text{Cl}}:$ , or ammonia, the nitrogen of which has a lone pair



The original distinction between valences as drawn by Werner, indicated by the terms "true" and "residual" valences, vanishes. Those within the first shell are, as a matter of fact, co-ordinate links, the outside atoms being held purely by electrostatic attraction. The distinction thus vanishes, and the valences that unite the groups to the central atom in Werner's co-ordination zone are identical co-valences, whether with an atom like chlorine or a radical like ammonia, the bond being formed by a lone pair of electrons.



A co-valency, or the number of co-valencies that an atom can form, is not conditioned by its position in the periodic table. In the aluminum hydroxide the aluminum is originally tri-valent, has six electrons obtained from the hydroxyl groups. It is, however, still unsaturated and requires eight for stability, and on aging it obtains two more as the hydrion dissociates from the hydroxyl group, and the oxygen attaches to the aluminum its lone pair. The aluminum inside the compound has thus increased its valency from three to four.

#### AGING AND STABILITY OF ALUMINUM SILICATE SOL

After the work of Hardy (3), it was believed and has since been frequently stated, that flocculation of colloid particles is due to the neutralization of the charge on a colloid by an oppositely charged ion. If it be assumed that the stability of a sol is due to the potential difference between two concentric layers, the sol will be more stable the higher the potential difference, and if the stability depends solely on the neutralization of the charge, the greater the adsorption of an oppositely charged ion, the greater the flocculation of the colloid. This view that the stability of the colloid is in some way connected with the cataphoretic potential is widely accepted, and the colloid on this viewpoint is least stable at its isoelectric point. Mattson (9), however, found that the flocculated colloid had in some cases a higher cataphoretic mobility than the sol from which it had been formed by coagulation with electrolytes.

The aluminum silicate synthesized as described was strongly positively charged, that is, the pH 4.5 was below the isoelectric pH of the compound, and it dissociated Cl ions. On suspension in barium acetate the pH came to pH 7.0, and excess of  $\text{Ba}^{++}$  was in the solution, so that actually, in studying the flocculation in barium acetate solution, we are dealing with the neutralization of a colloid that is negatively charged. The cataphoretic mobility in barium acetate could not, however, be determined, as the polarization at the poles of the cell makes such a determination impossible, but a pH 7.0 is considerably higher than the isoelectric pH of any aluminum silicate studied in this laboratory. It is therefore reasonable to suppose that the colloid was dissociating cations and would thus be negatively charged at such a high pH.

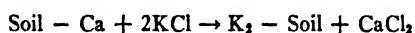
The difference in flocculation with the age of the colloid in a solution of barium acetate is very striking. The flasks were photographed by transmitted light and show clearly the difference in the stability with the age of the colloid, the oldest being the least stable, and a clear field is obtained with transmitted light, although it adsorbed the least amount of ions of opposite sign of charge. The increased flocculation could hardly thus be in any way due to neutralization of the charge by an ion of opposite charge (plate 1).

The increased coagulation is, however, due to the increased size of the particles, falling in the barium acetate under the action of gravity, for all the particles contain adsorbed barium in the external layer, and the particles themselves, once the barium has combined, have the same sign of charge. If the theory for aging is correct, and polymerization due to unsaturation results,

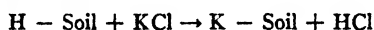
then this increased coagulation due to increased size of particles ought to be expected on Stokes' law, which states that if the force is gravity then  $V = Cr^2$ , the limiting velocity with which particles fall in a liquid of the same viscosity is proportional to the square of the effective radii. It should be noted that the  $Ba^{++}$  prevents the aging as judged from flocculation, so that a particle of Ba-colloid is actually smaller than that of a H-colloid.

#### BASE EXCHANGE

The phenomenon of base exchange was first discovered by Way (17), who percolated a solution of KCl through a soil and found that a differential adsorption takes place, the potassium being retained and another element, mostly calcium, coming into solution. The reaction may be expressed as:



Acid soils under similar circumstances exchange H ion for the  $K^+$  ion, and the reaction may be expressed as:



These reactions are termed base exchange reactions and have the advantage of simplicity of representation. The exact nature of the process, however, requires a great deal of elucidation. It has been supposed that the cations are held at the surface of a colloidal anion as in the Helmholtz double layer and can then be replaced by the cations of a salt without altering the stability of the particle.

Mattson (9) proceeded to elucidate base exchange reactions for materials other than soil, such as hydroxides of iron and aluminum, phosphates, and silicates. Basing the theory on the conception of amphoteric electrolytes, he determined in each case the pH at which the various compounds are isoelectric. Ferric hydroxide prepared from the chloride is isoelectric at pH 7.1 and the corresponding aluminum compound at pH 8.2. If these compounds are leached with an electrolyte, the pH of which is equal to their isoelectric pH, such as a solution of normal barium acetate, no base exchange occurs, or an extremely small amount. If they are treated with a salt solution the pH of which is higher than the isoelectric pH, base exchange occurs, but if treated with a solution the pH of which is lower than the isoelectric pH, anion exchange occurs, that is, the hydroxyl ion is replaced by the anion of the salt, and not the hydron by the cation of the salt. The isoelectric point thus assumes a new significance. It is not only the point of least stability of a sol, but it is a very significant point from the viewpoint of ion exchange. It is defined as the point at which the colloid is discharged, but characterized as well by the determination of pH at which the discharge occurs.

On the theory of amphoteric electrolytes, the isoelectric pH is given by

$$H = \sqrt{\frac{Ka}{Kb} Kw}$$

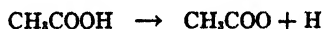
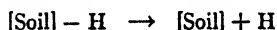
where  $Ka$  = dissociation constant of acidoid component;

$Kb$  = dissociation constant of basoid component;

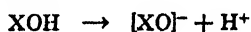
$Kw$  = dissociation constant of water

and the isoelectric pH will depend on whether the acid or basoid component is strongest, that is, has the strongest dissociation.

This theory of dissociation tendency, with suitable modifications, has a great deal in common with the usual theory of dissociation for the soluble electrolytes. The following exposition can be given, silicates being taken as an example. The anion of silicic acid is known as a sluggish ion with small mechanical mobility, although it may have a high electrical mobility. Its dissociation tendency is the same as that of any other acid, but the resulting anion does not become molecularly dispersed but remains bound in colloidal aggregates. If a particle of soil is placed in water, its dissociation tendency is made evident by the lowering of the pH, that is, it splits off H ions at its surface, which remain as the surface layer surrounding the anion, being held by electrostatic attraction. The charge of the colloid particle thus arises from dissociation, and an analogy can be made with any other acid thus:



If the colloid is amphoteric, that is, if it acts both as a base and as an acid, it must dissociate in two separate ways:

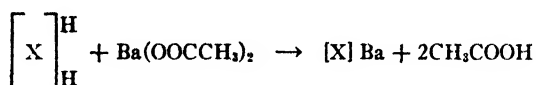


At the isoelectric point, if the charge arises out of dissociation of ions, the colloid is isoelectric because both dissociations are equal. It is important in this theory to realize the obvious fact that both dissociations are equal when there is no dissociation at all.

Chemical reactions take place between ions partially bound or free. By partially bound ions we mean those held at the surface of another ion by electrostatic forces, such as occur in a soil. These also occur in the solution of an electrolyte, where in the neighborhood of any ion there are more ions of an unlike charge than of the same charge. In the Werner salts, such as  $\left[ \text{Pt}(\text{NH}_3)_4 \text{Cl}_2 \right]$  where the chlorine is bound by co-valencies, it gives no test with silver nitrate but does if the compound is  $\left[ \text{Pt}(\text{NH}_3)_3 \text{Cl} \right] \text{Cl}$ . If base exchange is an ionic reac-

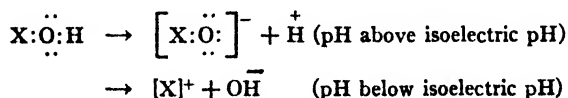
tion, as of course it is, and if a compound shows no base exchange capacity when leached with a solution the pH of which is the same as its isoelectric pH, then we are left with no alternative but to state that at its isoelectric point it is not ionized—it is neither cationic nor anionic. From the qualitative point of view, the test is identically the same as testing for  $\text{Cl}^-$  in Werner salts with silver nitrate. Since at this point there cannot be any ions dissociated from the colloid, it must, on the basis of the atomic theory, be a co-ordinate compound held by co-ordinate valencies, but we have to define the conditions under which a co-valency changes into an electro-valency.

In determining base exchange, such as with any colloid, by leaching with a normal solution of barium acetate, e.g.,



the reaction is thrown absolutely in one direction, by removal of the product of the reaction by leaching, so that the test for dissociation is extremely rigid, and yet neither ferric nor aluminum hydroxide, isoelectric at pH 7.1 and 8.2 respectively when prepared from the chlorides, shows any trace of base exchange when leached with a normal solution of barium acetate pH 7.0. There is, therefore, at this pH no cationic dissociation of these two colloids.

Co-valencies can, however, change into electro-valencies, that is, ions can dissociate from the compound under certain conditions, and for the systems studied the pH appears to be the determining factor. If an amphoteric colloid be treated with a salt solution at various pH values, it has been found by Mattson and Hester (10) that it dissociates H ions and lowers the pH of a salt solution which was originally above the isoelectric pH, that is, it dissociates in two ways:



but in the sense that not all of the surface layer splits off H or OH ions. One may speak, therefore, of partial, or degree of, dissociation, one of the ions resulting from the dissociation, the anion, being incapable in the case of soil of achieving the molecularly dispersed state. Depending on the character of the dissociation, it should be observed that the sign of the charge of the colloid particle changes. In a salt solution the dissociated ions  $\text{H}^+$  or  $\text{OH}^-$  are replaced by the ions of the salt, either partially or completely depending on the concentration of the salt.

Since the nature of the dissociation varies with the pH of the external medium, it would be expected that not only the nature but the amount or degree of dissociation should also vary. That is, if an amphoteric colloid is isoelectric at pH 6.0, by virtue of mass action, the dissociation of hydron from

the complex would increase the higher the pH or the lower the hydrogen-ion concentration of the liquid phase, and the greater would therefore be the base exchange, the higher the pH of the salt above the isoelectric pH. The reverse would be true of anion adsorption, that is, the lower the pH of the electrolyte, the greater would be the dissociation of OH ions and the greater the replacement by other anions. This has been proved for a number of hydroxides, and the results will be published later. In order to illustrate the point, however, a sample of electrodialyzed bentonite (1 gm.) was treated with 4.0 millimols of  $\text{FeCl}_3$ , and the pH was brought by the addition of alkali to the isoelectric pH 6.5. The sample was freed from the mother liquor and the base exchange of the ferric bentonite was determined on 0.5 gm. of the dried sample by leaching with solutions of barium acetate at pH 6.0 and pH 7.0, the barium being subsequently replaced by  $\text{NH}_4$  by leaching with normal ammonium chloride. At pH 6.0 the exchangeable barium was 0.192 m.e. per 0.5 gm., but at pH 7.0 it was 0.253. Since the isoelectric pH was at 6.5, if base exchange is regarded as a test for hydrogen ions there appears to be a marked contrast in the degree of dissociation at the isoelectric point for different amphoteric electrolytes. In the case of the hydroxides the minimum dissociation at the isoelectric point has a negligible value; with the silicated compounds it appears to reach a considerably higher value.

*The position of the hydrogen ion in base exchange*

Hydrogen occupies a unique position, as its kernel is composed merely of the nucleus with one planetary electron. Its stable shell in combination is not a group of eight, but a group of two, characteristic of the inert gas helium.

It has been found by Jenny (5) that with soil colloids the hydrogen ion is the most strongly adsorbed and the most difficult to replace. The order of adsorption of the various cations of the same valency at equivalent concentrations is as follows:



and for their release:  $\text{Li} = \text{Na} > \text{K} > \text{H}$ .

The strong adsorption of the H ion, which is attributed to its small volume, is surprising, for in all other branches of chemistry the high mobility of the H ion is attributed to its small volume, and in colloids, on aging, the whole phenomenon is interpreted on the basis of its mobility. Its strong adsorption and its mobility cannot therefore be attributed to one and the same cause, namely, its small atomic volume. It must be united by forces of a totally different nature from that which unites other cations, and although on the older theory of valency this cannot be explained, on the new it can, only one assumption being made, that is, that a chemical bond is a much stronger force than an electrostatic attraction between oppositely charged ions.

It has been shown that the surface, the seat of base exchange, in both hydroxides and silicates, consists of hydroxyl groups, or oxygen atoms with a lone

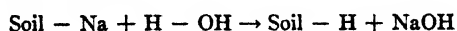
pair of electrons. The H ion dissociates little at the isoelectric point but the colloid cast as an acid if treated with a salt solution the pH of which is above its isoelectric pH, as a base if the pH is below its isoelectric pH, the dissociated ions forming a diffuse layer surrounding the colloid ion, and held there by electrostatic attraction. That is, the co-valent bonds change into electro-valent bonds at a pH removed from the isoelectric pH, but at two different points in the compound.

The strong adsorption of the H ion has been proved by treating a soil colloid with the hydroxides of lithium, etc., and subsequently releasing the adsorbed cation with an equivalent amount of acid. All soil colloids studied thus far have their isoelectric pH below pH 7.0, so that if placed in an alkaline medium, with a low concentration of H ions, the dissociation of the hydrion will be nearly complete, but the subsequent replacement of the H ion in the diffuse layer will depend on the concentration of the salt under equilibrium conditions. If this were not so, when a colloid is leached with a salt solution at any pH, the base exchange ought to go to completion whatever the pH, and since it does not, then replacement of the H ion of the diffuse layer is the secondary reaction, the primary reaction being the degree of dissociation of the  $H^+$  ions which is a function of the pH and of the pH alone.

If the colloid is represented by  $X : \ddot{O} : H$ , it will dissociate in an alkaline medium into  $\left[ X : \ddot{O} : \right] + H^+$ , the hydrion being replaced by a cation, but the oxygen retains the bonding pair of electrons. On the addition of acid the hydrion with no attendant electrons has in the lone pair of the oxygen the exact number that it requires to form a true chemical bond and reach the stable number of helium (2). No other cation can form a true chemical bond other than the hydrion. Its small volume has nothing to do with its strong adsorption, for it forms a true co-valent bond.

*The weakly adsorbed bases Na, K, Mg, Li, Ca*

Soil colloids in their natural state are always electronegative, that is, dissociating cations from the surface, and to determine their isoelectric point they must first be electro-dialyzed, which can be looked upon as a hydrolysis with the addition of external energy:



Since they are electronegative, the pH of the soil solution must be actually higher than the isoelectric pH of the soil colloid free from exchangeable bases. Under such conditions the soil colloid gives an acid dissociation, that is, the H ion dissociates, the co-valent bond between the oxygen and the hydrogen being broken, the oxygen retaining the bonding pair of electrons. The ions of the various cations that occur in the soil solution have the following system of planetary electrons: Na (2) (8); K (2) (8) (8); Li (2); Ca (2) (8) (8). On

the basis of the newer conception of valency, they cannot form a true bond, that is, a co-valent bond. Thus if sodium ion combines with the oxygen we have the structure (2) (8) (2), characteristic of highly dissociated salts like sodium chloride but not a true bond. Cations other than hydrion require eight electrons for stability, and with a single bond oxygen, they cannot obtain them. Because of the charge on the oxygen atoms, the cations are held, therefore, purely by electrostatic attraction of the negative colloidal ion. The force of attraction will vary with Coulomb's law, being inversely proportional to the square of the distance between charged spheres of opposite sign of charge. The distance between them, however, will depend not on the natural atomic volume of the ions, but on the hydrated ions, and since it has been shown by Wiegner (18) that the smaller the ion the greater the sheath of water molecules, the greater will be the distance separating the smaller ion from the colloid anion, and the smaller will be the force of attraction and the greater the dissociation. It may be remarked that an ion that is strongly adsorbed must be weakly dissociated, for the force of attraction is identically the same for both adsorption and dissociation from the colloid. At different concentrations, however, we are dealing with a force of repulsion between the cations themselves as well as a force of attraction with the colloidal anion.

#### *Influence of aging on the adsorption of barium by aluminum silicate*

As the aluminum silicate ages, its capacity for adsorption of barium diminishes, and table 1 shows that after the silicate has aged for 3 weeks, the amount of barium adsorbed by the oldest colloid was only 58 per cent of the adsorption capacity of the young freshly prepared colloid. Whatever the ion at the surface, the force of attraction at the surface is due to the lone pair of electrons shared between the ions and the surface oxygen. A molecular rearrangement with the transference of these electrons to saturate the aluminum diminishes the surface forces and hence the degree of adsorption.

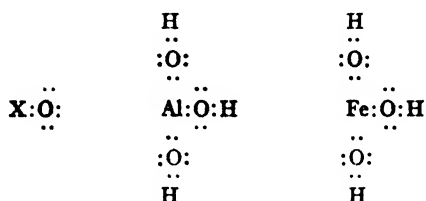
#### *Iron and aluminum in base exchange*

One gram of electro dialyzed bentonite in 1 liter of water was mixed with 4.0 millimols of  $\text{AlCl}_3$  and  $\text{FeCl}_3$  in a liter, and the pH of the supernatant liquid was brought to the isoelectric point pH 6.75 and pH 6.5 respectively. Complete adsorption of the iron and aluminum took place. The flocs were freed from the mother liquor, dried at  $65^\circ\text{C}$ ., and leached with normal ammonium chloride, but no trace of iron and aluminum could be found in the leachate. Although base exchange was not done on these precipitates, the cation exchange capacity of similar compounds was determined by Mattson (9) and had the following values in milligram equivalents per gram at pH 7.0: original bentonite, 1.002; bentonite  $\text{Al}_2\text{O}_3$  complex, 0.429; bentonite  $\text{Fe}_2\text{O}_3$  complex, 0.970.

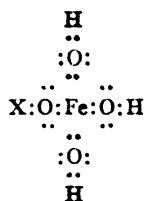
If these two ions do not take part in base exchange reactions, they cannot be at the surface of the colloidal anion but are blocked by some other ions. The atomic theory permits of an easy interpretation. On the assumption that

the bentonite has the same structure as that given the aluminum silicate, it can, as far as its surface is concerned, be represented by  $XOH$ , and since it is largely composed of aluminum and silica, the assumption is quite reasonable. Bentonite is an extremely strong acid, its acid dissociation is very high, so much so that some samples of bentonite are electronegative over a wide pH range with no isoelectric point. In acid solutions the concentration of H ions is so high that combination with iron and aluminum is impossible, but occurs at pH 6.5, when the concentration of H ions is so much lower that the H ion easily dissociates from the colloid, permitting combination with the iron and aluminum.

As the H ions dissociate, when the suspension is made alkaline, the oxygen atoms are left with the lone pair of electrons, with which the H ions previously combined. The chlorides of iron and aluminum also dissociate, the OH groups of the alkali attaching themselves to the iron and aluminum through the lone pair of the oxygen atom. The dissociated colloid and the iron and aluminum hydroxides may be represented as follows:



Both the atoms of iron and aluminum are unsaturated, for they have only six valence electrons, whereas for stability two more are required, which is the exact number the oxygen atom has to offer; the following type of compound, therefore, is formed:



The combination or the bond is co-valent, throughout the compound, that is, the pair of electrons constituting the bond come from one and the same atom, the iron and aluminum obtaining six electrons from the (OH) group and a pair from the oxygen of the dissociated colloid. The octet is thus completed, the iron and aluminum achieving stability by increasing their valency from three to four. They, however, combine "head on," as it were, with the bentonite, are blocked by hydroxyl groups, and can no longer take part in base exchange reactions. The cause of the formation of the bond is the same as with the H ion, except that for iron and aluminum a group of eight is required for sta-



bility, a co-valency of four, but the H ion achieves stability with two shared electrons, the helium number, whereas iron and aluminum have to obtain the octet of the other inert gases.

Upon the combination of the iron and aluminum hydroxides with the lone pair of the oxygen, the number of OH ions at the surface of the colloid particle are increased by two for each atom of hydrogen dissociated. Since base exchange occurs with the displacement of this hydrogen by other atoms, an increase of base exchange capacity would be expected, but actually a decrease at pH 7.0 is obtained. This is because the isoelectric points of the iron and aluminum bentonites are at such a high pH, pH 6.5 for the ferric compound and pH 6.75 for the aluminum compound, whereas the bentonite itself is electronegative, dissociating cations at all ranges of pH. Bentonite, a strong acid, will have a considerable dissociation at pH 7.0; ferric bentonite, isoelectric pH 6.5, will come next; and aluminum bentonite, isoelectric pH 6.75, will have the least dissociation at pH 7.0, and hence the least base exchange capacity, which is not, however, a linear function of the pH. Apart from the fact that different compounds have totally different isoelectric pH values, there is no means as yet of determining what proportion of co-valent and electro-valent fractions exist at the isoelectric point of each compound; the dissociation is at a minimum, but the absolute amount may be different for each compound.

#### THE CLAY COMPLEX

Robinson and Holmes (13) studied the composition of a large number of American soils and examined the hypothesis that the primary product of weathering is an aluminum silicate  $\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot \text{XH}_2\text{O}$  of the kaolinite type, and a hydrated ferric silicate, nontronite  $\text{Fe}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot \text{XH}_2\text{O}$ ; an excess of either sesquioxide or silica was found in most cases. G. W. Robinson (11), from the study of the composition of the clay fraction of Welsh soils, found that the data could, within limits, be represented on the assumption that the first product of weathering is a mixture of hydrated silicates of the general formula  $\text{R}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot \text{XH}_2\text{O}$ .

Considerable work in recent years has been done on the X-ray analysis of silicates by Bragg (2); on the clay fraction of the soil by Hendricks and Fry (4) and by Kelly, Dore, and Brown (6). The crystalline character of the clay fraction was demonstrated by comparison with the diffraction patterns of other known minerals, and some of the samples could be identified. Between these two conceptions, that of a colloid and the other a crystal, G. W. Robinson (12), in an interesting and critical discussion, finds us at a crossroad.

The distinction is not real, however, for the bonds in a colloid as in aluminum hydroxide, aluminum silicate, and from the evidence obtained from the iron and aluminum bentonite, in the natural bentonite as well, are co-valent bonds, arising out of one atom giving a lone pair of electrons necessary to form a single bond. To what extent the nature of the bonds can be determined by X-ray

analysis is very doubtful, for the silicates are very complex, and the interpretation of the data from this point of view is at this time only in its inception. In the case of the alkaline halides two types of structure occur, known as the rock salt and caesium chloride lattice. In the first type each sodium atom is surrounded symmetrically by six chlorine atoms and each chlorine by six sodium atoms; the second is similar except that the number is eight. This is taken as evidence that the force of attraction is electrostatic, with the sodium and chlorine existing as ions, so that there is no reason why a sodium atom should be more closely linked to one chlorine atom than to the other five. Such systems are, however, closely packed. If a system has an elaborate and open structure, occupying a large volume as in the soil colloid, it usually, as far as the evidence goes, points to co-valent bonds, but it is still crystalline in character. There is nothing incompatible with a crystalline character and dissociation in a polar medium. Sodium chloride is crystalline and dissociates in water; clay is also crystalline and dissociates in water. Although the degree of dissociation differs, its nature does not. An analogy can be drawn between strong electrolytes and weak electrolytes, such as sodium

TABLE 4

*Base exchange capacity of ferric silicate with barium acetate solution at different pH values*

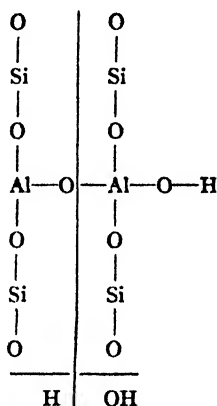
	pH OF BARIUM ACETATE		
	6.0	7.0	7.3
M e. barium adsorbed per gram. . . . .	0.627	0.981	1.141

chloride and acetic acid. An ionization of 100 per cent is almost certain for sodium chloride, an electro-valent salt, but acetic acid is co-valent with a small amount of electro-valent form. Replacing the H by sodium changes it into a strong electrolyte, and the molecular conductivities of sodium chloride and ammonium acetate are at moderate dilutions about 80 per cent of the values at infinite dilution, while that of acetic acid is only about 1 per cent.

For the interpretation of base exchange, X-ray analysis is not likely to prove very helpful, as base exchange is an ionic reaction that takes place between the colloid and a polar salt, and the determination of the isoelectric point and of the pH of the solution used for base exchange is much more important. The figures obtained for the base exchange capacity of a ferric silicate of  $\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3}$  ratio of 3.20, on being leached with barium acetate solution at various pH values, show the increase in base exchange capacity with increasing pH of leaching solution (table 4).

Further, as the colloid ages, as previously described, by continual polymerization of the molecules, its empirical composition, that is, the ratio of silica to alumina, remains the same, but its base exchange capacity will be different

because the number of molecules taking part in the base exchange reaction are fewer. The number of molecules can be increased by hydrolysis, and Mattson (9) found that hydrolyzing the Sassafras colloid with NaOH, increased its base exchange capacity 300 per cent. This is actually a reversal of aging, the polymerized molecule breaking thus:



An equal ratio of  $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3}$  is therefore no criterion of equal base exchange capacity, for in the soil polymerization and the reverse process of hydrolysis goes on, leading to profile development. The same colloid, however, whatever its degree of polymerization, will have the same isoelectric pH, for the surface bonds of the OH groups are the same whatever its age, and the isoelectric point is characterized by the pH when the colloid is either undissociated or dissociated amphoterically in an equal degree. The isoelectric point is therefore a much more fundamental point than the saturation capacity. The higher the base exchange capacity of any soil colloid as determined in the laboratory, the lower is its isoelectric pH and the higher the ratio of  $\frac{\text{Acidoid}}{\text{Basoid}}$  but this is only true because aging is a relatively rapid process, and drying the colloid probably accelerates the change. If they were of different age it is quite conceivable that there would be no correlation between the isoelectric pH and the saturation capacity, because of the different degrees of polymerization.

#### SUMMARY

The increased activity of the H ion during the aging of aluminum hydroxide and silicate has been studied, together with the flocculation in barium acetate solution and diminished adsorption capacity on aging.

Both the hydroxide and silicate were synthesized by hydrolysis of the chloride, the oxygen of the hydroxyl group or the silicate group combining with the aluminum ion through the lone pair of electrons of the oxygen. This is

by definition a co-valent bond, but the molecules of the freshly prepared colloids are unsaturated, the aluminum having only six shared electrons but requiring eight for stability. The hydrogen, because of its small volume and consequent high mobility, dissociates in a water suspension, and the lone pair of the oxygen are transferred to the aluminum, increasing the number of shared electrons by two, the aluminum becoming quadrivalent and saturated. This leads to continuous polymerization until all the material is exhausted and gives an unsaturated molecule. The freshly prepared sol is stable in a solution of barium acetate, but the 3-weeks-old sol flocculates rapidly because of the increased size of the particles. It is therefore suggested that a particle of Ba-colloid may thus be actually smaller than that of a H-colloid, provided the barium is added to the freshly prepared colloid.

The bearing of this on current theories of base exchange and the structure of clay is briefly discussed. The strong adsorption of the H ion is attributed to the fact that it is the only cation that can form a co-valent bond with two shared electrons, whereas the other cations must be held by electrostatic attraction, as they require a group of eight for stability. Iron and aluminum when combined with bentonite are not afterwards removed by leaching with ammonium chloride, the combination with the lone pair of the oxygen taking place with the unsaturated hydroxides.

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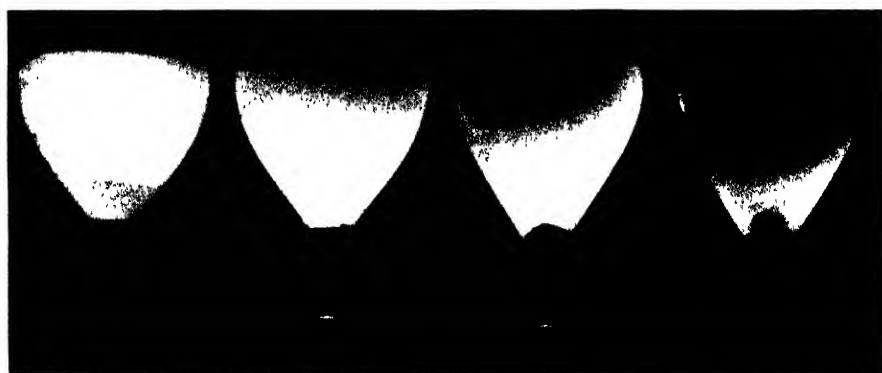
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### PLATE 1

#### EFFECT OF AGING ON THE STABILITY OF ALUMINUM SILICATE IN A SOLUTION OF BARIUM ACETATE

Age (reading from left to right): 0, 7, 14, and 21 days respectively





## Sven Odén

1888-1934

On January 16, 1934, Professor Sven Odén died, at the early age of 46. He was professor of chemistry at the Royal College of Technology in Stockholm, and in 1925 became chief of the Chemistry Department of the Central Institute for Agricultural Research, Experimentalfältet, Stockholm, Sweden.

Professor Odén's scientific activities were largely concerned with the sphere of colloid chemistry and its application to the science of the soil. His doctor's dissertation, "Der Kolloide Schwefel," is of considerable theoretical value. The method which he developed for following the rate of sedimentation in soil silting, using an automatically registering balance then calculating the frequency of the various sizes of the particles, is also generally known and highly valued. His contributions in the field of colloid chemistry must be considered of great and lasting importance. In his more recent investigations in the field of soil science, he paid special attention to the study of the acidity of the soil and its lime requirements, in which the electrodialysis method was extensively used and which, with the assistance of his staff, was technically perfected. Among other spheres of applied chemistry in which Odén exhibited his untiring genius for research may be mentioned his investigations on the chemistry of peat, cement, and concrete. His monograph, "Die Huminsäuren," presents a complete bibliography of the subject of the chemistry of the ill-defined and highly complicated group of humus compounds and an attempt to determine their chemical nature. In the field of plant physiology, the effect of light on the development of plants received Odén's attention; he also took considerable interest in practical experiments on the heating of soil by means of electricity and on the growth of plants by means of electric light.

Professor Odén's versatile gifts for research made him stand out as one of the leading personalities in modern agricultural research, from whom there was still much to be expected, and his untimely death is a great loss, particularly for his Scandinavian colleagues and friends.

H. LUNDEGÅRDH.





# THE CAUSE OF DECREASED NODULE FORMATION ON LEGUMES SUPPLIED WITH ABUNDANT COMBINED NITROGEN

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It has been shown repeatedly (1, 2, 4, 7, 23, 26) that the addition of rather large quantities of nitrates or other readily available nitrogenous salts will either prevent or greatly decrease nodule formation and nitrogen fixation on legume seedlings, depending upon the quantities added and conditions of growth. Under winter greenhouse conditions, where light is deficient and plant growth is slow, very small additions of nitrogen are sufficient to stop all nodule formation, while in the summer much larger rates of application are required to cause an appreciable effect. Giöbel and others have emphasized this point. It has also been proved by many workers, for example Giöbel, Prucha (16), and Hopkins, Wilson, and Peterson, that large applications of nitrogen do not prevent the entrance of the bacteria into the roots nor do they prevent the initiation of nodule formation in many cases. Such nodules, which may become of barely macroscopic size, practically always fail to develop further and may soon disappear if the soluble nitrogen supply continues abundant. Giöbel has also shown that if comparatively small quantities of soluble nitrogenous salts are added at regular intervals to the medium in which legumes are growing, nodulation will be depressed just the same as if added all at once. Furthermore, it was shown as early as 1900 by Hiltner (5) and since then by many others that if nitrates sufficient to stop nodule formation are supplied to a rather young plant, nodules may appear later in the life of the plant after the concentration of nitrate has been sufficiently depleted and that, too, without additional inoculation.

## SUGGESTED EXPLANATIONS FOR THE DEPRESSING EFFECT OF AVAILABLE NITROGEN ON NODULE FORMATION

In an attempt to account for the harmful effect of nitrates or other readily available sources of nitrogen on nodulation a variety of findings and suggestions have been offered by various investigators. Probably the most important of these are as follows:

<sup>1</sup> The writers wish to thank Dr. Dean Burk for many helpful suggestions given while this manuscript was being prepared and Messrs. H. J. Morris and Sam R. Hoover for assistance in carrying out some of the experimental work.

1. Hiltner (5) believed that nitrates strengthen the host plant and make it capable of resisting bacterial entrance. The whole phenomenon was explained on the basis of his immunity theory.

This idea is entirely theoretical and there is little or no evidence in support of it. Bacteria can enter roots even in the presence of excess nitrate and may initiate nodule formation.

2. The inoculating power of bacteria may be injured by the presence of nitrates.

Many of the earlier workers held this view, but it is now universally considered as not being in accord with the facts.

3. Göbel suggested that the plant can absorb and use nitrates much more quickly than the complex organic nitrogenous compounds fixed in the nodule, allowing the latter to accumulate and stop growth.

This is not a fundamental explanation for the phenomenon because where nitrates prevent nodule formation there is no nitrogen fixation and, of course, no accumulation of end products. A somewhat similar idea to that of Göbel's, commonly expressed, is that plants form nodules and fix nitrogen only in case they need it, but this is also no explanation for the cause of nodule failure.

4. Laurent (9) suggested that the nitrate reacts with the plant sap to form some compound toxic to bacteria.

Nitrate certainly does combine with the plant carbohydrates to form proteins as the end products, but these are not toxic to the bacteria. There is no evidence that toxic compounds are formed, at least none that are not also formed when atmospheric nitrogen is the source of this element.

5. Wilson (28) concluded from his divided root experiment that the effect of nitrates is local in character.

Present information partially supports this conclusion but, as will be pointed out later, the nutrition of the entire plant is involved. It should be emphasized in this connection that the maximum nitrate concentration which Wilson used was less than twice as great as one which permitted nodule production on both sets of roots. It would be expected in the light of carbohydrate-nitrate relations, to be discussed later, that higher nitrate concentrations (if not toxic) would have prevented nodule formation on both sides of the root system.

6. Strowd found very high concentrations of nitrate in root tissues grown in nitrate solutions and concluded that the concentration was sufficiently high to hinder the growth of the bacteria in the tissues and hence to stop nodule formation. Marshall (11) in earlier work came to a somewhat similar conclusion, suggesting that nitrates produce an osmotic state which hinders the development of the bacteria.

Göbel considered this idea at some length, and his view that Strowd's data do not justify his conclusions is undoubtedly correct. Nodule bacteria seem to be able to tolerate at least as high a concentration of nitrates as can legume plants.

7. Strowd also considered the possibility that the toxic action of nitrates may be due to their effect upon the hydrogen-ion concentration of the sap. Experimentally he observed little effect on the acidity and, hence, discarded the idea.

8. Mazé (13) suggested that the bacteria find sufficient nutrition outside the plant and do not enter it.

The answer to this suggestion is the finding of Göbel, Prucha, and others that nitrate applications do not prevent the entrance of the root nodule bacteria. Furthermore, Strowd makes the pertinent remark that if Mazé's theory is correct there should be no diminution in size of nodules in the presence of moderate amounts of nitrates; there should be either well-developed nodules or none at all.

9. Mazé in 1898 also suggested that the nitrate reacts with the sugar in the plant, leaving little for the bacteria. He believed that nitrogenous material is not harmful to bacterial development but that in the presence of excess nitrates the nitrogen combines with the carbohydrates formed in the leaves and little carbohydrate reaches the roots. Consequently, the root hairs do not lose any by diffusion, the bacteria in the soil are not attracted, and few,

if any, nodules form. These few remain small because they are deprived of carbohydrates. Weber's (26) recent studies led him to a conclusion only slightly different from that of Mazé, namely, that in the presence of excess nitrates there is no adequate carbohydrate nutrition for the bacteria and that the carbon-nitrogen ratio is not maintained sufficiently wide to permit the bacteria to carry on active nitrogen fixation.

In the opinion of the writers the carbohydrate deficiency idea is the only suggested explanation that is in agreement with the known facts, although neither Mazé nor Weber presented it in the true light. Mazé stressed the necessity for carbohydrate for bacterial entrance and growth, whereas Weber emphasized the need for a wide carbon-nitrogen ratio for bacterial growth and nitrogen fixation. These are not the primary functions of the carbohydrate. It is of much greater importance that a sufficient supply be available for the growth of the root tissue; otherwise it is impossible for the nodule tissue to form.

#### EFFECT OF SOLUBLE NITROGENOUS SALTS ON THE CARBOHYDRATE CONTENT OF ROOTS AND UPON ROOT GROWTH

Photosynthesis, of course, normally supplies the carbohydrates needed for respiration and growth of legume plants and for the bacteria living in the nodule. If plants (nonlegumes or nodule-free legumes) are grown in the presence of an insufficient nitrogen supply it is well known that they will accumulate carbohydrates, mostly as starch deposited in many places among the plant tissues. On the other hand, in the presence of an abundance of available combined nitrogen a rapid growth occurs, and the carbohydrate content in this case is kept low or is decreased if originally high. Growth occurs up to the point where carbohydrate becomes the limiting factor.

An extremely important point not heretofore stressed in this connection, which is the key to the whole discussion, is the fact that if an abundant supply of soluble nitrogen is given to a plant (legume or nonlegume) a greatly increased top growth and a comparatively small root growth result. This failure of roots to grow appreciably is due chiefly to lack of carbohydrates in the roots. There is such a mass of evidence in support of these ideas that it seems entirely unnecessary to present new experimental evidence. Miller (14) has discussed many phases of the subject at some length. The papers of Kraus and Kraybill (8), Gericke (3), Starring (22), Reid (17, 18, 19, 20, 21), and Müller (15), for example, show the importance of carbohydrates in root development and the unfavorable effect of nitrogen.

As a result of studies with nonlegumes, particularly barley and corn, Turner (25) concluded that "the increased ratio of tops to roots which results from increasing the amount of nitrate in the solution may be explained on the basis of increased use of carbohydrate in the tops because the greater nitrogen supply makes for greater growth. This results in a decrease in the supply of carbohydrate for the roots, which may bring about an absolute or a relative reduction of root growth." His experiments showed that nitrates actually increase (not

decrease) root growth where grown in pure culture with an adequate sugar supply.

The results of Fred and Graul (2), reported in part in table 1, together with calculations by the writers of the top-root ratios, bear directly upon the subject under discussion. In the case of alfalfa receiving only a little or no combined nitrogen the top-root ratio was about 1.0, whereas at a nitrate concentration

TABLE 1  
*Effect of nitrates on the relative growths of the tops and roots of alfalfa and vetch*

NITRATE N ADDED PER 100 CC. OF SOLUTION	DRY WEIGHTS		TOP-ROOT RATIO	NODULE OCCURRENCE
	Tops	Roots		
mgm.	gm.	gm.		
Alfalfa, uninoculated				
0	0.1	0.3	0.33	None
1	0.8	0.8	1.00	None
5	3.8	3.0	1.27	None
10	5.5	2.8	1.96	None
15	3.7	1.5	2.47	None
Alfalfa, inoculated				
0	0.3	0.2	1.50	Numerous
1	1.4	1.5	0.93	Numerous
5	3.9	2.5	1.56	Numerous
10	4.9	2.3	2.13	Few
15	5.6	2.5	2.24	None
Vetch, uninoculated				
0	0.6	1.8	0.33	None
1	3.4	2.0	1.70	One
5	5.0	4.2	1.19	None
10	7.4	2.5	2.96	None
15	3.9	1.2	3.25	None
Vetch, inoculated				
0	4.0	2.4	1.67	Numerous
1	4.9	6.7	0.73	Numerous
5	5.9	3.5	1.69	Few
10	7.4	2.5	2.96	Very few
15	4.0	1.5	2.67	None

sufficient to stop all nodulation the ratio was on the average 2.35. The figures for vetch showed a corresponding increase in top-root ratio from approximately 1.2 with little nitrogen to 3.0 where the nitrate application stopped nodule formation. With smaller nitrate applications the ratios were intermediate and nodule formation was an inverse function of the quantity of nitrogen added.

Welton and Morris (27) reported somewhat similar data for soybeans, the total weight of the roots of plants grown in a heavily manured soil being actu-

ally slightly less than on plants grown on a poor sand even though the weight of the tops was about 64 per cent greater in the former case. The numbers of nodules in the poor and rich soils were 379 and 62, respectively. Prucha (16) calls especial attention to the much better development of the root system of the Canada field pea grown in a nitrate-free solution in contrast to that grown in nitrate solutions.

It seems clear that nodules are produced only where root growth as compared with top growth is reasonably good.

A detailed consideration of why an abundant nitrate supply tends to reduce root sugar and in turn cause a wide top-root ratio scarcely falls within the realm of this paper. In fact, plant physiologists are not in entire agreement as to the correct explanation of the facts. Suffice it to say that it has been shown, at least in the case of the cotton plant that a sugar concentration gradient commonly exists between the leaves and the stems and roots. Furthermore, under conditions of abundant nitrogen supply carbohydrate is ordinarily the limiting factor in plant growth and, since all plant cells are consuming it, those farthest from the supply would be expected to receive the least and make the poorest growth, as Turner pointed out.

With this background of facts is it surprising that the addition of available nitrogen may decrease or stop nodule formation? Under exactly the same conditions the roots of the same plants make a negligible growth because of an insufficient energy supply. Nodule tissue may be considered, so far as this discussion is concerned, as root tissue slightly modified, and if the energy available is insufficient for root growth in the presence of nitrates it is obviously inadequate for the growth of nodules, located on the roots. With moderate nitrate applications some nodule tissue may form but under the same conditions some root tissue may likewise develop, the extent of development of both depending upon the carbohydrate supply, assuming that other growth conditions are satisfactory. It is an invariable rule that *nodules grow only where a sufficient supply of carbohydrates is present to allow at least a moderate root growth.*

When the question of why nodules fail to form in the presence of nitrates was first given consideration in this laboratory the advisability of making chemical analyses for sugars and starch in legume roots was considered, but the idea was set aside, chiefly for the reason that it was not believed that this method of attack would lead to conclusive results or furnish much new information. Although the total quantity of carbohydrates which reaches the roots in a given length of time may be greatly decreased by nitrogen additions the actual percentage of sugars in the roots of a plant growing in normal light will not necessarily be extremely low. Furthermore, any attempt to determine the effect of nitrates on root carbohydrates is greatly handicapped by the fact that even under reasonably constant growth conditions the normal diurnal and day to day variations in carbohydrates, especially of reducing sugars, may be rather large. Since we are dealing with a living system involving carbohydrate syn-

thesis on the one hand, and its utilization on the other, the quantity of readily available carbohydrates present at any one time represents only a small percentage of the total. This small percentage is, of course, subject to marked variations if either the rate of photosynthesis or utilization changes. While nitrate additions would undoubtedly tend to lower the carbohydrate content to some extent, an accurate determination of the nitrate effect, apart from other variations, would probably not be easy. The analytical difficulties are further intensified by the fact that the plant can easily change reducing sugars to starch or starch to sugars, as needed and where needed.

The data of Strowd, reported in table 2, are of interest in this connection. Reference should be made to his original paper for the experimental details.

It will be observed that Strowd found that at nitrate concentrations sufficient to stop nodule formation the concentration of reducing sugars in the roots was approximately half that of the check. Since the plant juice, even in the presence of nitrates, contained a fair concentration of reducing sugars

TABLE 2  
*Effect of nitrates in sand on the reducing sugar content of the root juice of soybeans*

NITRATE N ADDED TO 100 GM. SAND	FIRST EXPERIMENT (AFTERNOON HARVEST)		SECOND EXPERIMENT (MORNING HARVEST)	
	Nodules	Reducing sugar*	Nodules	Reducing sugar*
mgm.		mgm.		mgm.
0	Large, abundant	342	Large	220 0
2.5	Small, few	421	....	..
5 0	None	187	Very small	107 4
10 0	None	168	None	75 8
20 0	No germination			

\* Calculated as dextrose per 100 cc. of root juice.

Strowd was apparently not inclined to attach much importance to the idea that lack of carbohydrates was responsible for lack of nodules. He did not, however, entirely set aside the carbohydrate theory, concluding that "before any definite conclusion can be drawn in this regard, it will be desirable to determine whether or not the addition of sugar to the soil counteracts the depressing effect of nitrate." Göbel also rejected the carbohydrate idea on the basis of Strowd's findings.

Contrary to the views of Strowd and Göbel the writers consider that Strowd's data offer some evidence that nodules fail because of insufficient carbohydrate; at any rate the results are not contrary to that idea. The fact that the soybean roots contained reducing sugar is no proof that the root carbohydrate supply was adequate for the growth either of nodule or root tissue. There is certainly no reason to expect sugars to be absent under such experimental conditions, but rather they should be merely decreased as Strowd actually found in all cases except with the smallest rate of application of nitrate. Sugar must be present

in plant tissues continually to meet the needs for respiration or else the plant cells will soon die. After these respiration needs are met then if sufficient carbohydrate is present growth can take place, first largely in the leaves and stems and then in the roots. Not until the supply is adequate for a fair amount of root growth will nodule development occur. Apparently a slightly higher energy level is needed for nodule tissue growth than for root tissue growth, thus accounting for the fact that in the presence of an abundance of available nitrogen some root development may occur without nodules forming. The physical difficulty of transporting sugars into a nodule may be an adequate explanation, but it is also highly probable that the total energy requirements in the nodule for the respiration and growth of the bacteria and host tissue, for nitrogen fixation, and for protein synthesis, are greater per unit dry weight than the energy needs for a corresponding weight of root tissue.

We may summarize by saying that under constant conditions of growth the plant tends to establish a carbohydrate equilibrium. A change in photosynthetic activity or in the supply of available nitrogen may cause some change in the carbohydrate concentration, but except under rather extreme conditions ordinarily the variation is not over a wide range except temporarily. If the rate of photosynthesis is increased, in the presence of a normal nitrogen supply, then the carbohydrate concentration may also increase somewhat, but *it is the amount of plant growth that increases most and not the carbohydrate concentration.* On the other hand, if photosynthesis is decreased to the point where the supply is inadequate for respiration then of course the available carbohydrate concentration may drop to approximately zero. If the plants are maintained in normal light and the available nitrogen supply is made to vary, then an abundance of nitrogen causes a marked increase in top growth and a comparatively small root growth. We would expect some drop in sugar concentration in the roots, as Strowd found, but there should still be a fair concentration in view of the fact that such roots are still respiring and making some growth. It should always be borne in mind that this paper deals specifically with the latter set of conditions—normal light and high available nitrogen.

#### EXPERIMENTS TO DETERMINE THE EFFECT OF SUCROSE AND OF NITRATE ON NODULE FORMATION ON LEGUMES

The foregoing review and discussion suggest that a simple method of obtaining experimental data on the subject would be to add varying amounts of sugar and nitrates to the substratum in which legumes are growing and note the effect on nodule production. It is a well-established fact that the addition of sugar to plant roots usually causes a marked increase in root development and under some conditions also in nodule development, but very little is known about the combined effect of sugar and nitrates. Such experiments, alone, would never conclusively answer the question under consideration but the data should add an important link to the long chain of evidence already accumulated on the subject. Some work of this type is reported in the following.



The experimental method adopted was to determine the effect of sugar and of nitrate used singly and in combination on nodule formation on seedlings during short experimental periods. The cultures were not continued for longer periods because of the practical impossibility in sand culture of maintaining a constant sugar concentration or of preventing decomposition of the sugar by the nodule organisms, with the consequent presence in the substratum of various unknown end products. The use of solution cultures would be subject to the objection that under such conditions nodules do not develop normally and nitrogen fixation is usually slight.

Two preliminary experiments were made without success. In the first experiment sucrose and sodium nitrate were added to unsterilized sand, but fungi attacked the germinating seeds, especially where both nitrate and sucrose were added, to such an extent as to ruin the experiment. In another experiment sterile alfalfa seeds were planted in sterilized sand in bottles, fitted with cotton plugs, and kept under artificial lights. The temperature was excessive, and the germination irregular in the presence of sucrose, hence the experiment was soon discontinued. Two successful experiments, reported here as experiments 1 and 2, are described in detail in the following.

*Experiment 1.* In this experiment 4,200-gm. portions of washed nitrogen-free sand were placed in glazed earthenware jars and moistened with 675 cc. of Thornton's (24) nitrogen-free culture solution. After being covered with paper the pots were sterilized, and 105 sterile alfalfa seeds were placed on top of the sand and covered with 300 gm. of sterile dry sand. The required amounts of sterile sucrose and nitrate solutions were added, likewise 10 cc. of a heavy inoculum of a young culture of alfalfa bacteria, and sufficient distilled water to bring up to weight. The following day the pots were placed in a special artificially lighted apparatus, to be described elsewhere (10), which has given excellent results in the growing of plants. The light source was ten 500-watt Mazda lamps and one glass mercury vapor lamp per total bench area of 25 square feet, giving a light intensity of about 2,000 foot candles. The temperature of the sand in the pots was maintained between 20 and 27°C. by means of a water bath. Aseptic precautions were taken in starting the experiments, but the pots were exposed to the air after planting and no further precautions were taken against contaminations other than to avoid unnecessary handling. The work was done in a basement room which was very free from dust. No difficulty was experienced from contaminating organisms either in this experiment or in experiment 2, although undoubtedly the sand was not kept entirely free from foreign organisms. The average number of seeds per pot which germinated was about 85 out of a total of the 105 seeds planted. The extremely weak and abnormal plants, which on the average amounted to about 12 per pot, were discarded in all cases. The nodule counts, made 7, 8, and 14 days after planting, are recorded in table 3.

It will be observed that the concentration of nitrate used was barely sufficient to retard early nodulation but not to prevent it, and that after 14 days it was

about 76 per cent as good in the presence of nitrates as on the check plants. Sucrose, alone, also markedly retarded the production of nodules on alfalfa, as shown by the first count, but the effect had been largely overcome by the

TABLE 3

*Effect of potassium nitrate and sucrose, used singly and in combination, upon nodule production on alfalfa seedlings*

	PER CENT OF PLANTS WITH NODULES AFTER			AVERAGE NUMBER OF NODULES PER PLANT AFTER		
	7 days	8 days	14 days	7 days	8 days	14 days
Untreated.....	58.2	68.0	99.5	1.05	1.68	5.57
Potassium nitrate (20 mgm. N).....	28.1	25.6	97.5	0.33	0.33	4.26
Sucrose (1 gm.).....	19.6	50.6	94.9	0.25	0.88	4.76
Potassium nitrate (20 mgm. N) plus sucrose (1 gm.).....	40.0	74.7	100.0	0.67	1.63	5.72

TABLE 4

*Effect of varying concentrations of potassium nitrate and sucrose upon the nodulation of alfalfa seedlings*

SUCROSE (GM. PER POT)		POTASSIUM NITRATE (MG. N PER POT)						
		0	2 5	5	10	20	40	80
0	{	0 83	0.63	0.23	0.18	0 08	0 05	0.00
		4.52	3 54	4.72	3.71	3.17	1.02	0.34
0.125	{	1.16	0.59					
		3.42	4 93					
0.25	{	0 72		0.58				
		2.87		4.26				
0.5	{	1.17			1.16			0 00
		5 53			6.64			0.27
1.0	{	1.25				0.37		
		5.29				5.30		
2.0	{	1.31		0 70			0 38	0 04
		3.56		4.48			4 12	0 51

Upper figures—average number of nodules per plant after 8 days.

Lower figures—same after 12 days.

following day. It is a well-known fact, confirmed by the experiments reported here, that in the presence of added sugars seed germination is frequently retarded and the growth of the young seedlings hindered. Where both sugar and nitrate were added there was some retardation in germination, but to a

lesser extent than with sucrose alone. The nodule counts in this case, made on the seventh day, were higher than where either sugar or nitrate alone was added but slightly less than on the check. The counts made the following day showed that the sugar had entirely overcome the harmful effect of nitrate on nodulation. It is clear, then, that in this experiment even though sucrose, used singly, decreased early nodulation it was nevertheless able to counteract the harmful effect of nitrate where used together with it. This is direct evidence for the idea that lack of available carbohydrates is responsible for decreased nodulation in the presence of soluble nitrogen.

*Experiment 2.* In the second experiment the methods used were the same as in the first experiment except that varying concentrations of sucrose and nitrate, added singly and in combination, were used. The results are given in table 4.

It will be observed that at the first counting of nodules increasing concentrations of nitrate had decreased nodule production proportionately up to the highest concentration, where no nodules had formed. The various applications of sugar alone, with the exception of one treatment, had increased the number of nodules formed in 8 days. In the previous experiment sugar alone showed a marked retardation of nodulation at the end of 7 days but the effect was nearly overcome by the 8-day period. In this experiment, where counts were not made until the 8-day period, four out of five pots showed more nodules than the check. The combined application of sugar and nitrate usually resulted in the formation of more nodules than where only nitrate was used although in only one out of eight pots was the number of nodules as great as on the check plants at the 8-day period.

The data obtained on the twelfth day show the continued retarding effect of nitrate on nodulation except at the lowest concentrations. Sugar, alone, caused a slight increase in two cases and a decrease in three others. These plants grew more slowly than those in the check pots, showing that sugar under these conditions was somewhat harmful to growth. The use of sugar together with nitrate counteracted the effect of the latter to such an extent that nodule production was approximately the same as on the check plants, except at the largest rate of application of nitrate.

#### DISCUSSION

The experimental evidence presented here shows that regardless of the fact that sucrose, alone, retarded germination and plant growth it did to a marked extent counteract the harmful effect of nitrates on nodulation. If the retarding effect of sugar had not been so marked it is reasonable to suppose that it would have had an even greater effect in counteracting the nitrate effect. It is also probable that the sucrose entered the roots so slowly under sand culture conditions that the concentration of the sugar in the root sap was not markedly increased and hence produced less effect than would have been observed in solution cultures.

Although the writers consider that their data lend support to the carbohydrate hypothesis, the results do not, of course, prove it but merely serve as an additional bit of the evidence, all of which is apparently in harmony with the idea. It is again desired to emphasize that nodule formation involves the rapid growth of tissue, and in searching for the reason for nodule failure we must find the reason for the failure of root tissues to grow. When conditions are such as to prevent rapid root growth it cannot be expected that nodule development will continue unchecked. Since we know that *an excess of assimilable nitrogen greatly retards root growth as compared with top growth and may even practically stop it temporarily because the carbohydrates are used almost quantitatively in the tops, it is scarcely necessary to look further for an explanation of the failure of nodules to develop on these same roots. The nodule bacteria play an incidental rôle in the phenomenon.*

After this article was ready to be sent to the publishers the recently published paper of Hopkins and Fred (6) came to the attention of the writers. Reference to this article will show that in most respects the conclusions presented agree with those presented here except that emphasis is somewhat differently placed. Hopkins and Fred emphasize the necessity for a sufficient concentration of carbohydrate to permit the bacteria to proliferate and incite nodule formation, whereas we believe that the bacteria play a very secondary rôle and that the carbohydrate is needed mostly for plant tissue development, whether it be nodule tissue or normal root tissue. Experimentally, the initiation of nodule formation alone was considered in this laboratory, whereas at the Wisconsin station more emphasis was placed on the distribution and size of nodules and on the quantity of nitrogen fixed after a long growth period.

#### SUMMARY

A review of previous work and suggestions, dealing with the cause of the depressing effect of available nitrogen on legume nodule formation, is given.

Calculations made from previously published data show that the top-root ratios of legumes grown with varying amounts of available nitrogen increase with increase in nitrogen supply. Under similar conditions there is a decrease in the quantity of reducing sugars in such roots.

Experiments are reported showing that sucrose, added to sand cultures of alfalfa, tended to overcome the injurious effect of nitrate on nodule formation except at the highest concentration of nitrate used. This beneficial effect of sucrose was observed regardless of the fact that where used alone it retarded germination and early plant growth.

It is believed that the evidence from all the experimental work so far reported proves beyond a reasonable degree of doubt that decreased nodulation in the presence of soluble nitrogenous salts is due to inadequate carbohydrate supply in the roots. The effect on the bacteria themselves plays a very secondary rôle in the phenomenon. Where nitrogen is abundant the carbohydrate synthesized is used for top growth, and little is available for the growth of roots or

nodules. Nodules grow only where enough carbohydrate is present to allow at least a moderate root growth.

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## PLANT FEEDING BY BRIQUETTE FERTILIZATION

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Two major problems in economic and efficient fertilization of sugar cane have been under study for several years at this experiment station. One problem relates to the matter of excessive leaching of applied potash to the soil, whereas the other embraces the intricate subject of soil phosphate fixation. In this contribution a discussion is offered upon a type of fertilization which appears to offer, among other advantages, a means of minimizing excessive leaching of potash and of materially reducing fixation in the soil of applied phosphates.

Statements appear in the literature supporting claims by investigators purporting to show that some plant roots have the ability to feed upon nutrients in the solid phase. Carefully planned experiments are in progress at this time in which the habits of the cane plant, in this respect, are under observation.

The author has examined submerged wooden water pipes in which the flow of water was hindered by a penetration of plant roots. The pipes later became entirely useless as a result of the complete choking of the bore. Abandoned macadam and concrete roadways will usually furnish a good illustration of the inherent forces present in germinating seeds whereby the solid paving material becomes parted or shattered in order that growing root systems may find a path to food and moisture. Cases of rock cleavage by the growing roots of various plants have also been observed. Since plant roots thus strike through rigid material which is met in natural environment, they should penetrate equally well into artificially placed solid material containing food substances which may be acceptable or required. It became apparent, then, that were a source of plant food to be intimately incorporated with a cementing medium, properly diluted, and, were such a mixture hardened by mechanical compression or natural cementation, it should furnish a means of fertilization in which loss of soluble nutrients would be greatly reduced and phosphate fixation perhaps entirely overcome as set forth.

Laboratory experiments were conducted in the preparation of firmly setting cement and fertilizer briquettes. In order to reduce chemical interaction of cement and fertilizer salts and later to minimize leaching of soluble constituents, a briquette was finally produced containing only, one soluble ingredient (potassium chloride). A formula found satisfactory at that time consisted of the following mixture:



Dried blood.....	50 parts by weight
Bone meal.....	50 parts by weight
Raw rock phosphate.....	50 parts by weight
Potassium chloride.....	50 parts by weight
Portland cement.....	125 parts by weight
Sand.....	50 parts by weight
Water.....	Sufficient

It was found that in the course of a few hours the briquette made by this formula would readily solidify in initial set and that in a day or two following it would have become sufficiently firm and dry for experimental use. When immersed in water the briquette retained its solid form without disintegration, but as was to be expected, there was a slow but constant loss of potash to the surrounding water. In other respects, however, the product appeared entirely satisfactory.

In order to ascertain the value in service of such a cemented mixture it was decided to make a "field" test of the briquettes in an attempted fertilization of water lilies under the bed of a flowing stream. The conditions of this test, we believed, were sufficiently severe to furnish direct evidence as to the value of the scheme, particularly regarding the ability of a plant to utilize fertilizer in such a form, even when submerged adjacent to running water. A section of the stream was found (about 30 feet in width) where pond lilies were already growing on either side. The condition and size of the plants at either bank were very nearly alike. All of the plants on one side were treated as follows: In an arc of about a 1-foot radius, up stream from the main plant stem, three depressions were made, small stones, soil, and sand being removed for a depth of about 10 to 12 inches. This treatment was given when the water was very low and stream flow practically negligible. A single briquette, weighing about 1 pound, was placed in the bottom of each depression, and the stones and soil were returned until all the excavations had been filled and smoothed off level with the bed. The plants on the other side of the stream were not treated in any manner. In the course of about 5 to 6 weeks the leaves of the plants in the treated area had grown larger than those having received no treatment. In the course of about 3 months the contrast in leaf size and number of leaves, as well as color, marked between the treated and untreated lilies. The treated plants produced many more flowers of a considerably larger size individually than those growing in the other area. After about a year had elapsed, at a period of very low water, an attempt was made to remove an entire root system of one of the treated plants. The attempt was not entirely successful, but we did find that the roots of the plant had grown to, and surrounded, the briquettes with a basket-like weave of comparatively large roots, and in the place formerly occupied by the briquette a mass of very fine roots had grown. Nothing remained of the original briquette except a sandy residue. Upon pulling up other root clusters in the treated area we were enabled to shake all of the disintegrated residue of the briquette out of its basket-like enclosure by striking the whole assembly against the side of a rock.

The experiences gained from this experiment led to further trials of the briquette fertilization scheme in pot cultures with soil, eucalyptus and papaya trees being used this time as indicator plants.<sup>1</sup> Briquettes were prepared according to a formula similar to the one described, with the exception that no bone meal was used. The average dry weight of the briquettes was 414 gm. each, the plant food content being calculated as 1.4 per cent nitrogen, 4.25 per cent phosphoric acid, and 7.0 per cent potash.

Eight concrete tubs were selected having a capacity each of about 50 pounds of soil. Preparatory to filling the tubs, the soil was thoroughly mixed, screened, and then quartered. The experiment was laid out into four sets, in duplicate, on October 27, 1931, as follows:

- Set 1: Control, no treatment; each pot planted to one papaya seedling.
- Set 2: Six briquettes placed at the bottom of each pot and planted to one individual papaya seedling.
- Set 3: Control, no treatment; identical to set 1, except planted to one young eucalyptus tree.
- Set 4: Six briquettes placed in the bottom of the pot and then planted to one eucalyptus seedling.

In all cases, plants were selected of the same age and as nearly as possible of the same size and development.

The soil selected for this experiment was particularly weak in potash. About 2 months after being planted, the untreated eucalyptus developed a dark reddish coloration of all leaves and stems. Eucalyptus growing in pots containing briquettes were free from this symptom of disorder. Six months later a decided response to the briquette treatment had become evident in every case. The treated plants exhibited a more abundant growth and a better color and were taller and more bushy than the controls (pl. 1).

After the root systems of both eucalyptus and papaya had been given opportunity to develop in the presence of the briquettes for a period of about 1 year, a root assembly of each variety of tree was carefully removed from the pots and washed free of soil. The roots of the papaya had not grown to or around any of the briquettes, but in the case of the eucalyptus tree, fine roots had surrounded the briquettes and at various points along their outer surfaces root terminals had drilled into the firm mass for some distance. Figure 1 of plate 2 shows two such briquettes which were cut free from the main root system so that root attachment might be shown.

The development and growth of the trees were allowed to continue until the roots of the treated eucalyptus were severely pot bound. Figure 2 of plate 2 illustrates the relative growth of treated and untreated plants at the close of the experiment (18 months).

In the early stages of growth the response of the papaya to briquette fertilization was not pronounced. Later, however, the treated trees showed marked gains over the untreated. In the case of eucalyptus, response was immediate,

<sup>1</sup> This work was assigned to Mr. Carl W. Nesbitt, Staff Chemist, and was thereafter conducted by him.

and as the trees developed the comparison between treated and untreated plants became more manifest and striking.

Although it is true that this experiment gave indications that certain plants may feed upon briquettes in dry land practice, still there are certain marked disadvantages in using Portland cement in the preparation of fertilizer briquettes. The first objection is a chemical one. The highly alkaline cement reacts with many of the more common soluble and partially soluble fertilizer materials and so limits the range of fertilizers which may be employed. This fact renders it practically impossible to use soluble fertilizers in the fabrication of a Portland cement type of briquette. The second objection is found in the high calcium content of the cement, a factor which is not always desirable for certain plants or for field use in some localities.

The study, was continued,<sup>2</sup> following the completion of the experiment described in the foregoing. The first objective was to develop a means of substituting in the briquette mixture satisfactory cementing materials other than Portland cement.

A number of briquettes were prepared in which plaster of Paris was used as the binding and hardening medium. In order to arrest loss of nutrients by solution from this porous type of briquette they were coated (after setting and drying) with a heavy application of paraffin wax. This latter treatment, of course, would add to the handicap imposed upon any root system in extracting nutrient substances from the concentrated source of supply.

With sugar cane as an indicator in a series of 50-pound pots, the plaster of Paris briquettes were employed as an only source of applied fertilizer<sup>3</sup> in a low grade, unproductive soil.

The experiment consisted of:

- (a) No treatment; duplicate pots.
- (b) One briquette per pot weighing about 1 pound, but the entire briquette reduced to powder and applied to the soil.
- (c) One briquette in each pot, placed near the bottom of the soil container.

In this experiment it was planned to take note of any differences which might develop as a result of introducing the fertilizer in one large unit, protected against excessive loss by seepage, as opposed to the same materials applied to the soil following as nearly as possible the practice used on a field scale. At 3 months of age the cane growing in the pots containing the solid briquettes showed a noticeable improvement over those growing in the pulverized briquette treatment. A greater number of suckers had grown from the stools, the leaves of the plants were greener, wider, and longer, and in all respects these plants, nourished by solid briquettes, showed a marked growth and vigor over the other two sets (pl. 3, fig. 1).

<sup>2</sup> Mr. Paul L. Gow, staff chemist, conducted this experiment.

<sup>3</sup> Briquettes were so fabricated that one would contain an equivalent amount of fertilizer per unit quantity of soil as would be applied in the general practice current throughout the Islands in fertilizing this crop.

Following these observations, laboratory studies on the preparation of new types and kinds of briquettes for experiment in the field were made. The encouraging progress in the study rendered it advisable at this point, we thought, to summarize results and to outline the project on a more comprehensive basis. The following points were noted:

1. To be produced in large quantities, a cheap method of manufacture must be found. Having in view a system similar to that used on the mainland in briquetting coal dust, we looked into that process with the view of employing it with our materials if it were found feasible.

2. It appeared of advantage to confine fillers and binders to a class of compounds which would:

Be inexpensive and readily obtainable locally.

Not react chemically with the fertilizer constituents in a manner resulting in a weakening of cementation, nor should these materials impair the fertilizers from functioning to their full capacity. However, we *were* seeking a chemical combination of fertilizer constituents and conditioning substances which would enhance cementation without detriment to the product. (It will be shown later how this objective was realized.)

3. The field of briquette usefulness appeared to include all fertilization in general, but it was particularly adapted to areas of boggy land, marshes, and infertile districts subject to excessive rainfall. Briquettes appeared also to offer advantages in fertilization of soils where fixation of applied phosphates may be pronounced.

4. The increase in cost by briquetting operations must be offset by distinct advantages accruing from the use of this method of plant nutrition.

It was planned to take up, first, the search for a satisfactory binder and filler, and thereafter, with the aid of a hydraulic apparatus for compressing briquettes, to carry on experimental studies in the fabrication of a more generally useful and practical product.

In a search for a binder to supplant Portland cement and other bulky compounds it was found that a granular, dry sodium silicate (of special formula) could be utilized in the preparation of an excellent briquette. It was found, too, that a native black basaltic rock, when combined with the silica binder, produced a briquette which would accomodate almost any type of soluble or insoluble fertilizer, either singly or in combination. (As a matter of fact the more popular or common soluble fertilizers will usually produce a more satisfactory briquette of the silica binder type than will a mixture consisting mainly of insoluble materials.) By compressing such mixtures under hydraulic pressure a very firm and stable briquette could be prepared. Such briquettes were found to resist disintegration in water over a long period of time and to give up their soluble fertilizer salts at a low rate. A typical mixture consists of the following materials:<sup>4</sup>

Ammophos 13/48 .....	24.3 gm.
Nitrate of potash .....	21.2 gm.
Powdered sodium silicate (spec. formula) .....	11.2 gm.
Ground basalt .....	56.7 gm.

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<sup>4</sup> Using this briquette, one per foot of line, lines 5 feet apart, is equivalent to an application of 100 pounds N, 225 pounds P<sub>2</sub>O<sub>5</sub>, and 175 pounds K<sub>2</sub>O per acre.

<i>Ratio:</i>	
Filler to fertilizer— 1 to 1	Hydraulic pressure . . . . . 4,000 pounds
	Weight of briquette . . . . . About 115 gm.
	Size of briquette . . . . . { Cylinder Diameter 2 inches Height 1½ inches

About 300 of these briquettes were prepared and have been placed in a field experiment with sugar cane on the Island of Kauai.<sup>5</sup>

In calculating costs of this new type of briquette we found that the use of the granular silicate increased the outlay above fertilizer per acre by about \$17. The cost of the filler (basaltic rock) was practically nil. We felt it necessary, therefore, to devise another type of briquette in which the expensive silicate could be eliminated.

It was found that the acid constituents in the fertilizer salts, when brought in contact, under hydraulic pressure, with moisture and powdered basaltic rock of one type very common in the Islands, liberated, upon dehydration, sufficient silicic acid from the basalt to bind the mass thoroughly. A typical formula of this newest type of briquette<sup>6</sup> follows:

Ammophos 13/48 . . . . .	24.3 gm.
Nitrate of potash . . . . .	21.2 gm.
Ground Moililii basalt . . . . .	91.0 gm.

<i>Ratio:</i>	
Filler to fertilizer— 2 to 1.	Hydraulic pressure . . . . . 4,000 pounds
	Weight of briquette . . . . . About 140 grams
	Size of briquette . . . . . { Cylinder Diameter 2 inches Height 1¾ inches

A large replicated pot experiment is now being prepared with sugar cane as the crop and in which the latest briquettes are to be employed for comparison with equal quantities of granulated briquette materials, with regular fertilizer mixtures of equivalent plant food content, and with no treatment whatever.

In other field, forest and pot experiments now under way it is our purpose to gather information which may throw light on the type of briquettes which should be employed to lend themselves readily and efficiently to the feeding habits of the crop for which they may be used. The root habits of various crops must also be studied in relation to their ability to surround and penetrate the briquettes. The size of the briquette is also an important factor, as well as its rate of disintegration. For perennial plants such as shrubs, bushes, and

<sup>5</sup> At the request of, and under the direction of Mr. W. W. G. Moir, agricultural technologist, American Factors, Ltd.

<sup>6</sup> Briquettes made from this formula and used 1 foot apart in 5-foot lines would fertilize 1 acre at the same rate of applied N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O as that resulting from the use of the silicate mixture previously described.

trees, a briquette should be employed which will slowly and continuously give up small amounts of nutrients over a period perhaps of several years, whereas with sugar cane a briquette must be employed which will be completely disintegrated and will have discharged its nutrients prior to the time that the crop is to be ripened and harvested. The matter of soil type must also be taken into consideration in this study.

Further study indicated that the size, shape, and consistency of briquettes may be varied to suit any particular need. Thus, to produce a rapidly disintegrating product, the amount of filler may be reduced to very small proportions or it may be eliminated entirely.

Should the requirements call for a very hard briquette, as may be needed for plants, shrubs, and, particularly, trees, the amount of basaltic silicious filler (and binder) may be increased several times over the ratio appearing in the formulas given. Such a practice automatically dilutes the active fertilizer constituents, produces a larger and harder briquette, and also slows down the rate at which the fertilizer may diffuse, be dissolved, or be extracted by penetrating roots from the cemented mass.

For needs lying within these two extremes the amount of binder and size of briquette may be adjusted similarly.

Figure 2 of plate 3 shows three briquettes, each carrying the same amount and weight of fertilizer, but combined with variable amounts of basaltic filler.

#### CONCLUSION

The briquetting of fertilizers appears to offer a means of supplying a plant with insoluble or soluble nutrients under conditions where ordinary fertilizer mixtures would prove prohibitive or impractical.

The scheme is suggested as an expedient in overcoming heavy potash leaching or excessive soil phosphate fixation.

Briquette fertilization is under trial in Hawaii for starting young trees on barren slopes. At the time of planting, a few briquettes of slowly disintegrating character are introduced in the post hole near the roots of the sapling. The objective is to sustain and boost the growth of the young tree until its root systems are capable of carrying on and have reached lower zones of more abundant moisture. It has been found practicable adequately to fertilize aquatic plants by introducing fertilizer briquettes under the bed of a flowing stream. Briquette fertilization compares favorably with normal practice in the few experiments so far conducted. In one case (described in this paper) the centralization of nutrient supply in briquettes resulted in a more favorable response by the plant than was realized from the same materials rendered granular and then applied in the usual manner.

#### SUMMARY

Mechanical and chemical means of briquetting fertilizers are described. The development may be adjusted to meet the requirements of almost any type

of soil, crop, or plant. The advantages of employing briquetted fertilizer over regular practice in special cases are discussed. Basic formulas are given which may be varied to suit the needs of the experimenter. Responses are described in plant growth by the briquette fertilization in a stream bed of running water. Photographs are offered, together with discussion, bearing upon results obtained by briquette fertilization of trees in dry land practice and with sugar cane in concrete tubs.

#### PLATE 1

##### EFFECT OF BRIQUETTE FERTILIZATION ON PAPAYA AND EUCALYPTUS SEEDLINGS

Photographed June 30, 1932, 8 months after planting. A very poor soil from Manoa Substation, Field 9G was employed. The soil is extremely low in  $P_2O_5$  and  $K_2O$  and has never been fertilized. Reaction = 5.4 pH

FIG. 1. Indicator plant: Papaya seedlings

FIG. 2. Indicator plant: *Eucalyptus ovata*

Control

Treatment

Control

Treatment



Control

Treatment

Control

Treatment



## PLATE 2

FIG. 1 Cement type of slowly disintegrating fertilizer briquette, showing root attachment and incipient formation of surrounding basket-like "root weaving" 1 year after eucalyptus had been planted. Most of the root systems were removed from the attached portions before photographing.

FIG. 2 Indicator plants. Papaya seedlings and *Eucalyptus orata*. The last set of plants used in the initial experiment, photographed March 28, 1933, 18 months after planting. The first set was harvested when 12 months old, October 24, 1932.



Treatment    Control  
Papaya

Treatment    Control  
Eucalyptus

## PLATE 3

FIG. 1. Briquette fertilization of sugar cane; 3 months after planting

X = no treatment

F = Granulated briquettes incorporated with soil

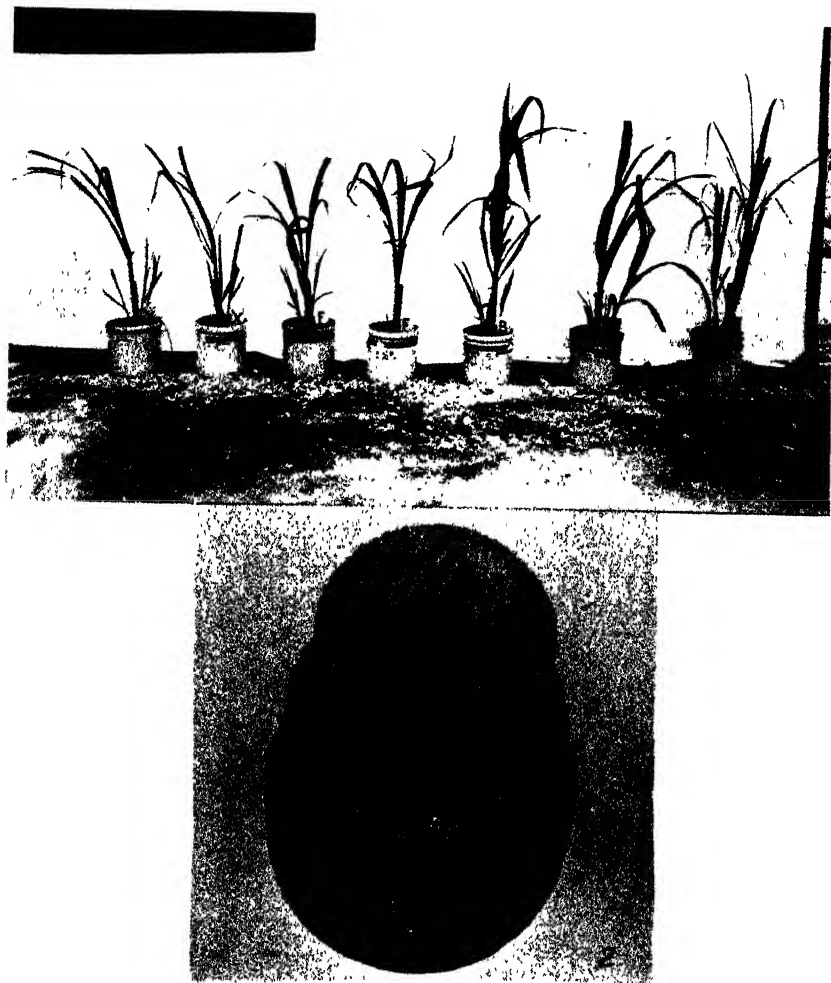
B = Solid briquettes (not granulated) prepared with plaster of Paris and coated with a heavy layer of paraffin. Comparable and identical in nutrient supply to the "F" treatment

FIG. 2. Types of briquettes

The small briquette (top) consists of fertilizer only (no tiller). Weight = 63.75 gm  
Size = diameter, 4.2 cm, height, 2.66 cm

The next larger and lower briquette carries equal quantities of tiller and fertilizer  
Weight = 116.8 gm. Size = diameter, 5.16 cm, height, 2.92 cm

The bottom (largest briquette) is a mixture of 2 parts tiller to 1 of fertilizer. Weight = 176.4 gm. Size = diameter, 6.04 cm, height, 2.86 cm





# THE INFLUENCE OF THE pH OF A CULTURE SOLUTION ON THE ASSIMILATION OF AMMONIUM AND NITRATE NITROGEN BY THE TOMATO PLANT<sup>1</sup>

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In an earlier publication (3) the influence of the pH of a culture solution on the rates of absorption of ammonium nitrogen ( $\text{NH}_4\text{-N}$ ) and of nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) by the tomato plant (*Lycopersicum esculentum*, Mill.) was reported. As it is quite conceivable that not only the pH of the culture medium, but also the concentrations of  $\text{NH}_4\text{-N}$ , of  $\text{NO}_3\text{-N}$ , or of other nitrogenous constituents of the tissues of the plants used for the absorption tests might have influenced the rates of absorption of either or both forms of nitrogen, data will now be presented giving the proportions of certain nitrogenous fractions in the plants used for these absorption tests. The relationships between the rates of absorption of  $\text{NH}_4\text{-N}$  and of  $\text{NO}_3\text{-N}$  and the concentrations of these forms of nitrogen in the plants will be pointed out, and an attempt will be made to correlate the observed relationships with the assimilation of nitrogen by the plants, as indicated by certain nitrogenous fractions in aqueous extracts of the plant tissues, by the amounts of protein nitrogen in the tissues, and by the green weights of the plants.

Many experiments have been reported on the effect of the pH of soils or of culture solutions on plant growth, but only a few typical ones with higher plants grown in solution or sand cultures which have a direct relation to this study with tomatoes will be mentioned. Mazé (9), Hutchinson and Miller (7), and others have reported greater yields of plants grown in culture solutions including  $\text{NH}_4\text{-N}$  when  $\text{CaCO}_3$  has been added to the solutions than when it has been withheld.

Prianischnikow (17) obtained greater yields of sugar beets when they were supplied with a pH 7.0 culture solution including only  $\text{NH}_4\text{-N}$  than when they were supplied with the same solution including only  $\text{NO}_3\text{-N}$ . In contrast, when the pH of the solution was lowered to 5.0 or 5.5, greater yields were obtained when the plants were given  $\text{NO}_3\text{-N}$  than when they were given  $\text{NH}_4\text{-N}$ . Furthermore, the yields from the solutions including  $\text{NH}_4\text{-N}$  were greater at pH 7 than at pH 5, and the yields from the solutions including  $\text{NO}_3\text{-N}$  were greater at pH 5 than at pH 7. Dikussar (5) using continuously renewed solutions confirmed these results.

Mevius and Engel (10), from experiments with maize plants, concluded that the rate of penetration of ammonia into the root cells was increased as the pH of the solution was increased, but they stated that protein was not necessarily formed if abundant carbohydrates were not available. Their work included analyses of the plants for several nitrogenous fractions.

Tiedjens and Robbins (21) grew several different crop plants in solutions of different pH and used either  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , or both in the complete culture solutions, which were applied by the sand culture method. From the growth of the plants and from analytical data they concluded that ammonia was most efficiently assimilated at pH 7 or above, and that

<sup>1</sup> Journal Series paper of the New Jersey Agricultural Experiment Station department of plant physiology.

<sup>2</sup> It is a pleasure here to acknowledge the valuable suggestions received from Dr. G. T. Nightingale concerning analytical procedures employed.

ammonia, although absorbed, was not assimilated at pH 4. They also found that nitrates were most efficiently assimilated at pH 5, but that ammonia at pH 8 was assimilated more rapidly in the tomato plant than were nitrates at pH 4 to pH 5.

Pirschle (14), in confirmation of earlier results, found that at strongly acid and at alkaline reactions, the growth of plants supplied with  $\text{NH}_4\text{-N}$  was less than that of plants supplied with  $\text{NO}_3\text{-N}$ . He noted that the pH optimum was about 5.5 to 6.5, within which range the differences between plants grown with  $\text{NH}_4\text{-N}$  and plants grown with  $\text{NO}_3\text{-N}$  were least.

Beaumont (1) found that  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{NH}_4\text{Cl}$ , and  $(\text{NH}_4)\text{H}_2\text{PO}_4$  were toxic to tobacco grown in culture solutions, although the young plants grew well for a while if  $\text{CaCO}_3$  was added to the solutions. From analyses of the plants it was suggested that the toxicity of the ammonium salts was due to poor assimilation of the ammonium ion and consequent improper metabolism.

From the aforementioned experiments it can be concluded that the pH of the culture medium has a pronounced influence upon the assimilation of both  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  by higher plants, although not all of the investigators mentioned have agreed on the pH range at which the assimilation of  $\text{NH}_4\text{-N}$ , especially, has been most efficient. This is not surprising, considering the relationship which the type of plant and its supply of carbohydrates (8, 10, 12, 14, 16, 21) may have to the assimilation of nitrogen.

#### METHODS OF EXPERIMENTATION

##### *Method of Growing Plants and Testing Absorption*

The technique employed in growing the plants and in testing their rates of absorption of  $\text{NH}_4\text{-N}$  and of  $\text{NO}_3\text{-N}$  was described in the preceding paper (3). As was mentioned there, the plants used for these absorption tests, as well as others grown in corresponding solutions, were harvested within a few hours after each absorption test was completed and were immediately prepared for analysis. From the end of the absorption interval until the time of harvesting, the plants were grown in the same solutions as those used preceding and during the absorption tests.

##### *Preparation of Tissue for Analysis*

The plants to be analyzed were first separated into roots and tops. The latter were further separated into two fractions, (a) stems and petioles and (b) leaf blades. The stem and petiole fractions included all tissue to which the blades were not directly attached, whereas the leaf blade fractions included only the blades. The roots were well rinsed with a stream of tap water and then with distilled water and were dried between paper towels until adhering water had been removed.

The green weights of the separated fractions were all recorded and the tissues were then very finely minced. Duplicate 100-gm. aliquots of the stem and petiole fractions and duplicate 60-gm. aliquots of the blade fractions were immediately placed in an oven at  $100^\circ\text{C}$ . This temperature was decreased to  $80\text{--}85^\circ\text{C}$ . in about an hour, before the tissue had dried thoroughly. After being dried for 3 days at the lower temperature, the tissue was weighed, ground in a

drug mill, and used for total nitrogen analyses. The percentage dry matter data, as well as total nitrogen analyses of duplicate aliquots, showed that the aliquoting was very uniform.

One 100-gm. aliquot of each portion of minced tissue was immediately placed in boiling water and was subsequently extracted by wringing and washing. A comparison of this method of extraction with that in which sand is first used to grind the tissue is to be published (4). Extraction of all the samples was usually completed within 12 hours after the tissue had been boiled. Tests with replicate aliquots of minced corn tissue showed that once the fresh tissue had been boiled, extraction even after 48 hours gave the same results for the various fractions as extraction immediately after boiling.

#### *Chemical Methods*

*Ammonia nitrogen.* The liter extract obtained from each 100-gm. portion of tissue was boiled and the coagulable nitrogen removed as previously described (4). Duplicate 100-cc. aliquots were then taken, and ammonia nitrogen was removed by a modification of the method of Sessions and Shive (19). Eighth-normal NaOH was substituted for the  $\text{Na}_2\text{CO}_3$  and NaCl originally suggested, with aspiration at room temperature for a period of at least 16 hours. This modification was adopted as a result of the work of Tiedjens and Robbins (21), who found that a fraction which they called "combined ammonia" could be removed by aspiration with NaOH after the unmodified (19) aspiration with  $\text{Na}_2\text{CO}_3$  and before the addition of Devarda's alloy for the reduction of nitrates. This fraction, which they sometimes found to be even greater than the ammonia removed by  $\text{Na}_2\text{CO}_3$ , obviously should not be included with the nitrate nitrogen, as would occur if the unmodified (19) procedure were used. By the use of NaOH in the aspiration for ammonia, this "combined ammonia," if present, was included in the ammonia fraction in the data reported here. Sessions and Shive (19) have shown that little breakdown of certain amino acids occurred when these were aspirated with 0.125*N* NaOH and Devarda's alloy. Furthermore, the comparatively small amounts of ammonia nitrogen reported in the following tables do not indicate the inclusion of any considerable amount of nitrogen which should have been reported in some other fraction. The terms, "ammonia nitrogen," and " $\text{NH}_4\text{-N}$ ," are used interchangeably.

*Nitrate nitrogen.* After removal of the ammonia nitrogen from the aforementioned aliquots, 2.5 gm. of Devarda's alloy was added and aspiration continued for another period of 16 hours according to the method outlined by Sessions and Shive (19). However, the results obtained from the extracts of the stems, which contained an exceedingly large amount of nitrate nitrogen, were not compatible with the results from the other fractions in that the total inorganic nitrogen from the aqueous extract was too high. Therefore, analyses for nitrate nitrogen in all of the stems were repeated on the dried tissue. Because of the buffering action of the plant material, nearly twice as much NaOH was used as in the original determinations. The results for nitrate nitrogen which appear in table 4 are those obtained from the dried tissue.



*Amide nitrogen.* Immediately after the removal of the aforementioned aliquots, the remaining volume of each extract (800 cc.) was evaporated to 200 cc. and divided into duplicate 100-cc. aliquots, to each of which was added enough conc.  $\text{H}_2\text{SO}_4$  to give a 5 per cent solution. Suitable tests showed that this concentration prevented any significant changes in the distribution of nitrogen in the concentrated extracts, some of which were necessarily left in this stage while others were being subjected to the treatments which followed.

These concentrated aliquots were hydrolyzed for  $2\frac{1}{2}$  hours, cooled, and neutralized with 30 per cent NaOH. The ammonia then present, including both ammonia nitrogen and amide nitrogen, was removed by aspiration as described in the foregoing, and the previously determined ammonia was deducted. Since these tissues contained abundant nitrates, the use of  $\text{H}_2\text{SO}_4$  in the hydrolysis was essential, as has been shown by Vickery and Pucher (24) and Chibnall and Miller (2).

*Humin nitrogen.* After the solutions had been aspirated for the removal of amide nitrogen, they were neutralized with conc.  $\text{H}_2\text{SO}_4$ , made slightly acid with one drop in excess, and evaporated to 100 cc. The slight precipitate formed was filtered off and washed on No. 1 Whatman filter paper. Total nitrogen determinations on this precipitate by the method next described showed the presence of a negligible quantity of nitrogen (about 0.001 per cent in the roots and stems and 0.005 per cent in the leaves). Therefore, no values are reported for humin nitrogen. According to the procedure of fractionation employed, the result of this omission of the humin fraction was to add the humin nitrogen of the aqueous extract to the protein nitrogen. The latter fraction being many times as great, no appreciable error was introduced.

*Total soluble nitrogen.* The humin-free filtrate was made up to a volume of 100 cc., and an aliquot of 25 cc. was taken from each duplicate for a total nitrogen determination. To the result was added the nitrogen previously removed in the amide determination. In as much as the extracts included nitrates, the modified Kjeldahl-Gunning method for including nitrates, as outlined by Ranker (18), was used. The solution was kept slightly acid during evaporation, a precaution necessary to avoid the loss of nitrogen.

*Total amino nitrogen.* Aliquots of the humin-free filtrate were used for this determination. The method of Van Slyke (22) was employed, and it was found to be advantageous to substitute a burette taken from a micro-size apparatus for the large burette found on the macro-size apparatus employed. This permitted the use of large aliquots for analysis and an accurate estimation of small quantities of gas. In as much as this fraction included some nitrogen properly placed in the next fraction, the results of the total amino determinations do not appear in the tables.

*Basic nitrogen.* A 50-cc. aliquot from each of the duplicates of the humin-free filtrate was also removed and the basic nitrogen precipitated with phosphotungstic acid precipitating solution prepared according to the directions of Osborne and Harris (13). Fifteen cubic centimeters of the precipitating solu-

tion were sufficient for these aliquots. The solution including the precipitate was allowed to stand, with occasional stirring, for several days, and was then filtered off on No. 42 Whatman filter paper. All the precipitate was removed from the beaker with the aid of a moistened filter paper, but no washing was attempted, for this often disperses the precipitate into the filtrate. Analyses of the filtrate for nitrates showed that even when the precipitate was not washed a negligible adsorption of nitrates, at least, occurred.

*Basic-free amino nitrogen.* The filtrate from the basic precipitate was neutralized with 30 per cent NaOH, made slightly acid with a drop of conc.  $\text{H}_2\text{SO}_4$ , and evaporated to a volume of 50 cc. An aliquot of 25 cc. was sometimes used for another total nitrogen determination to check the amount of basic nitrogen obtained by analysis of the precipitate, but the remainder of the filtrate was available for a second amino determination carried out as described in the foregoing. Only the values for basic-free amino nitrogen appear in the tables.

*Protein nitrogen.* Total nitrogen was determined on aliquots of the dried tissue by the same method (18) as that employed for total soluble nitrogen. The difference between the total and total soluble (non-coagulable) nitrogen is regarded as protein nitrogen (11).

*Soluble organic nitrogen.* This fraction was calculated by subtracting the sum of the ammonia and nitrate determinations from the total soluble nitrogen.

*Other nitrogen.* This was calculated by subtracting the sum of the soluble nitrogen fractions reported from the total soluble nitrogen determined analytically. Consequently, it includes nitrogen not recoverable by the methods employed as well as the result of experimental error in the other determinations.

## EXPERIMENTAL RESULTS

### *Absorption Data*

Descriptions of the appearance of the plants at the time of harvesting have been given in the preceding paper (3). Some of the data, however, have been selected from the tables in that article and these are repeated in table 1, in the order which is adapted for comparison with the data presented in the following tables. In table 1 are listed only the data on absorption by those plants for which the analyses of plant tissue are reported. Absorption data in table 1 are the averages of triplicate cultures each including three plants, whereas the green weight figures are the averages of five cultures when the plants were 41 days old, and three cultures when the plants were 52 days old. The culture solution used included approximately 75 mgm. per liter of both  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ .

### *Composition of Roots in Relation to Absorption*

The several nitrogenous fractions obtained from the aqueous extracts of the roots made at the time of each harvest are listed in table 2, together with the average green weight of the roots per plant. The nitrogen in each fraction is expressed as a percentage of the green weight of the tissue in every case. The

values given for plants in each of the treatments at the age of 41 days are based on an extract from a composite sample of tissue from five cultures, each including three plants. Three of these cultures had been used for the absorption test on the day of the harvest. The values for the plants at the age of 52 days are based on tissue from three cultures, each including three plants. All three cultures had been used for the second absorption test on the day of the harvest.

TABLE 1

*Rates of absorption of nitrogen by plants used for absorption tests*

Absorption expressed as mgm. N absorbed per 100 gm. green tissue in 6 hours from a solution including equal proportions of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ .

	AGE OF PLANTS					
	41 days				52 days	
pH of solutions . . . . .	4.0	5.0	6.0	7.0	4.0	7.0
Average green weight per plant in gm. . .	53.0	55.1	64.6	57.8	139.0	117.8
$\text{NH}_4\text{-N}$ absorption . . . . .	3.4	4.2	4.6	6.6	2.2	5.0
$\text{NO}_3\text{-N}$ absorption . . . . .	4.8	5.9	4.1	3.0	7.6	8.4
Total N absorption . . . . .	8.2	10.1	8.7	9.6	9.8	13.4

TABLE 2

*Nitrogenous fractions in roots of plants used for absorption tests*

Nitrogen expressed as percentage of tissue on green weight basis

	AGE OF PLANTS					
	41 days				52 days	
pH of solutions . . . . .	4.0	5.0	6.0	7.0	4.0	7.0
Average green weight of roots per plant in gm. . . . .	7.4	7.9	11.5	11.0	17.2	17.5
Total soluble N . . . . .	0.100	0.117	0.132	0.142	0.105	0.139
Soluble organic N . . . . .	0.045	0.060	0.059	0.060	0.060	0.072
Basic-free amino N . . . . .	0.020	0.027	0.034	0.038	0.023	0.040
Basic N . . . . .	0.020	0.024	0.020	0.016	0.027	0.019
Amide N . . . . .	0.005	0.007	0.006	0.006	0.006	0.011
Other N . . . . .	0.001	0.002	-0.001	0.001	0.005	0.001
Ammonia N . . . . .	0.013	0.018	0.031	0.042	0.014	0.039
Nitrate N . . . . .	0.043	0.040	0.042	0.040	0.031	0.028

In as much as the data included in table 2 are too extensive and diversified to be considered as a unit, certain sections will be selected and treated separately.

*Nitrate nitrogen.* To correlate, if possible, the rates of absorption of  $\text{NO}_3\text{-N}$  with the concentrations of  $\text{NO}_3\text{-N}$  in the root tissue of the plants used for the absorption tests, figure 1 is presented. It is based on data taken from tables 1 and 2. It should be emphasized that the absorption rates were calculated on the basis of the green weight of the whole plants, not of the roots.

The diagram shows that there was no correlation between the rates of absorption of  $\text{NO}_3\text{-N}$  from the solutions and the concentrations of  $\text{NO}_3\text{-N}$  in the roots. It is evident that the variations in concentration of  $\text{NO}_3\text{-N}$  in the roots of the plants 41 days old with variations in pH of the culture medium were not significant, despite marked differences in the rates of absorption of  $\text{NO}_3\text{-N}$  from solutions of different pH.

Moreover, the difference between the concentration of  $\text{NO}_3\text{-N}$  in the roots of the plants 52 days old grown in the pH 7 solution and that of those grown in the pH 4 solution was not significant. However, the concentrations of  $\text{NO}_3\text{-N}$  in the roots of plants grown in solutions of corresponding pH decreased significantly as the plants grew older.

The fact that no more  $\text{NO}_3\text{-N}$  accumulated in the roots of plants which were absorbing  $\text{NO}_3\text{-N}$  at a high rate than in those which were absorbing it at a much

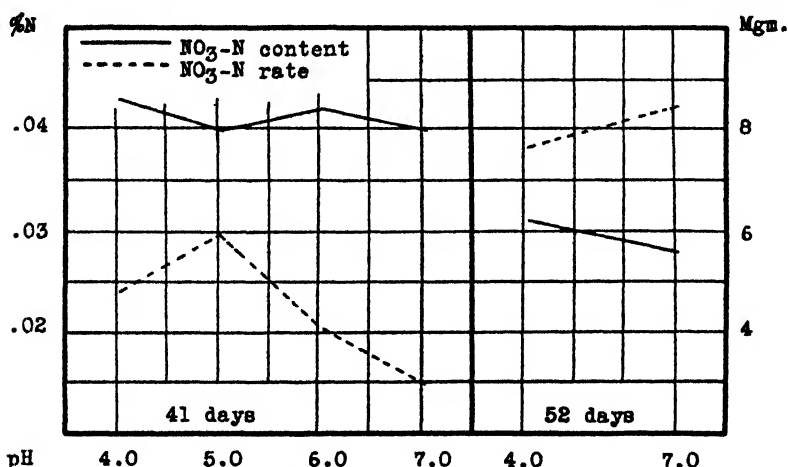


FIG. 1. NITRATE NITROGEN IN TOMATO ROOTS, EXPRESSED AS PERCENTAGE OF GREEN TISSUE, AND RATES OF ABSORPTION OF  $\text{NO}_3\text{-N}$  FROM CULTURE SOLUTIONS, EXPRESSED IN MILLIGRAMS OF NITROGEN ABSORBED PER 100 GM. OF GREEN TISSUE IN 6 HOURS

lower rate suggests that such an accumulation was prevented either by more rapid translocation or by more rapid reduction of the  $\text{NO}_3\text{-N}$  when the rate of absorption of  $\text{NO}_3\text{-N}$  was high than when it was low. From a consideration of the data on the composition of the tops as given in the following, the latter alternative appears to be the more probable one.

Mention should be made of the high concentration of  $\text{NO}_3\text{-N}$  in the roots of the plants as compared with that in the external solution, despite which high rates of absorption of  $\text{NO}_3\text{-N}$  occurred. A concentration of 0.040 per cent is equivalent to 400 mgm. of  $\text{NO}_3\text{-N}$  in 1,000 gm. of green tissue, of which only about 5 per cent is dry matter. Hence, the concentration of  $\text{NO}_3\text{-N}$  in the tissue was more than 400 mgm. per liter of water. In contrast, the concentration of  $\text{NO}_3\text{-N}$  in the external solution was approximately 75 mgm. per liter.

*Ammonia nitrogen.* Figure 2 presents the rates of absorption of  $\text{NH}_4\text{-N}$  in relation to the concentrations of  $\text{NH}_4\text{-N}$  in the roots of the plants, the method of plotting being the same as in figure 1. Figure 2 clearly shows that the pH of the culture medium had a pronounced influence upon the concentration of  $\text{NH}_4\text{-N}$  in the root tissue and that this influence was directly related to the rates of absorption of  $\text{NH}_4\text{-N}$  from the culture solutions. For example, the concentration of  $\text{NH}_4\text{-N}$  in the roots of the plants 41 days old grown in the pH 7 solution was three times that in the roots of plants of the same age grown in the pH 4 solution. At the same time, the rate of absorption of  $\text{NH}_4\text{-N}$  was about twice as high from the pH 7 solution as from the pH 4 solution.

In other words, the more rapidly  $\text{NH}_4\text{-N}$  was absorbed by the plants, the more was present in the roots per unit of plant tissue. The ratio of absorption

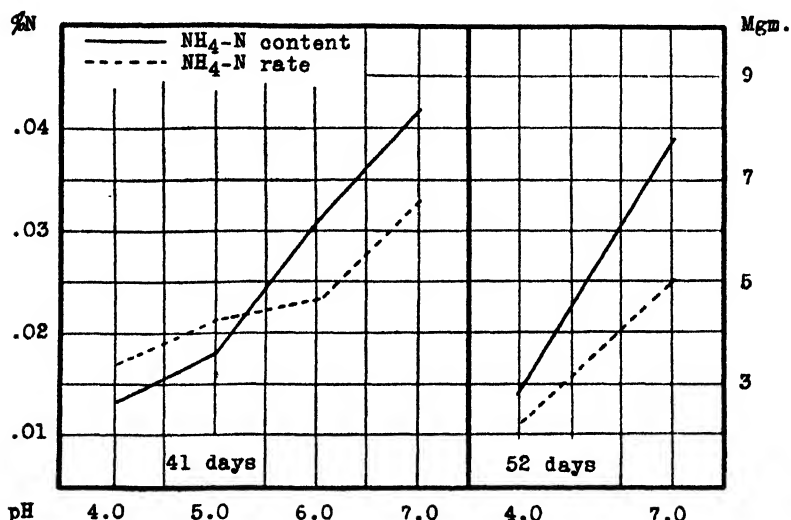


FIG. 2. AMMONIA NITROGEN IN TOMATO ROOTS, EXPRESSED AS PERCENTAGE OF GREEN TISSUE, AND RATES OF ABSORPTION OF  $\text{NH}_4\text{-N}$  FROM CULTURE SOLUTIONS, EXPRESSED IN MILLIGRAMS OF NITROGEN ABSORBED PER 100 GM. OF GREEN TISSUE IN 6 HOURS

to concentration in the roots was not a constant for plants grown in solutions of different pH. Indeed, that should not be expected, since it is quite probable that some of the  $\text{NH}_4\text{-N}$  in the roots arises from the reduction of nitrates absorbed. Eckerson (6) states that reducase is present in the tops and roots of the tomato in about equal proportions.

It is noteworthy that the high concentration of  $\text{NH}_4\text{-N}$  in the roots of the plants grown in the pH 7 solution did not prevent a very rapid rate of absorption of  $\text{NH}_4\text{-N}$  from the external solution. The ratio of the concentration of  $\text{NH}_4\text{-N}$  in the root tissue of these plants to that in the external medium, calculated in the same manner as given for  $\text{NO}_3\text{-N}$ , was of the same order as the  $\text{NO}_3\text{-N}$  ratio.

Figure 2 brings out one other point, that the concentration of  $\text{NH}_4\text{-N}$  in the roots did not increase as the plants grew older, within the limits of this experiment. Instead, the concentrations of  $\text{NH}_4\text{-N}$  in roots of plants grown in solutions of corresponding pH values were quite alike at 41 days and at 52 days.

*Basic-free amino nitrogen.* To show that the accumulation of  $\text{NH}_4\text{-N}$  in the roots of the plants grown in the high pH solutions did not result simply in a piling up of inorganic nitrogen within the roots, figure 3 is presented, the data for which were taken from table 2. Figure 3 shows the close connection between the concentration of  $\text{NH}_4\text{-N}$  in the tissue and the concentration of amino

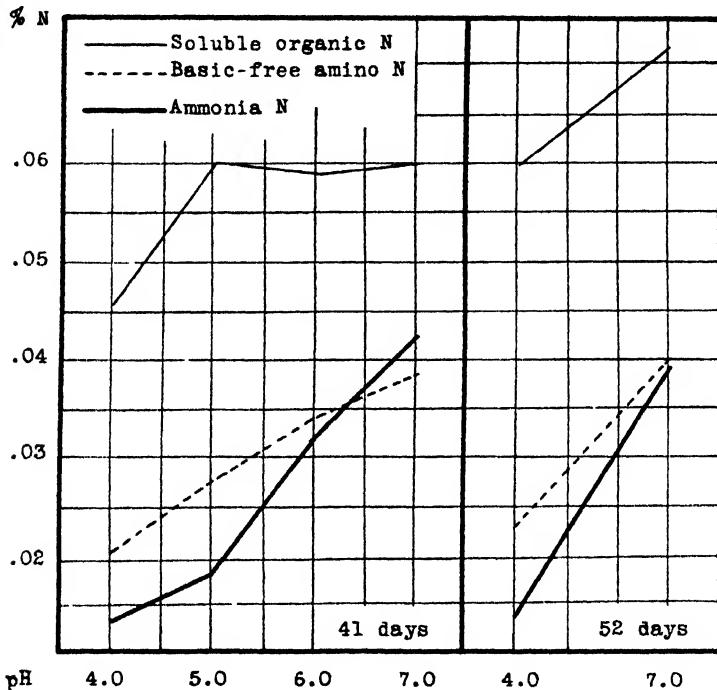


FIG. 3. AMMONIA NITROGEN, BASIC-FREE AMINO NITROGEN, AND SOLUBLE ORGANIC NITROGEN IN TOMATO ROOTS, EXPRESSED AS PERCENTAGE OF GREEN TISSUE

nitrogen in the tissue. In every case, a high concentration of  $\text{NH}_4\text{-N}$  was accompanied by a high concentration of amino nitrogen. This suggests a rapid formation of amino nitrogen in the roots from the  $\text{NH}_4\text{-N}$  present, the amount of amino nitrogen formed being greatly increased by the presence of increasing amounts of  $\text{NH}_4\text{-N}$ .

*Soluble organic nitrogen.* The graph of figure 3 which represents the percentages of total soluble organic nitrogen in the tissues of the roots shows, in comparison with the other fractions represented, that the amount of soluble organic nitrogen present was, in a measure, related to the concentration of

amino nitrogen present. However, as table 2 shows, other fractions than amino nitrogen comprise a considerable portion of the soluble organic nitrogen, consequently the total organic nitrogen present was not always proportional to the amount of amino nitrogen included in it.

In order to show the percentage of the total soluble organic nitrogen comprised in each fraction, the data of table 3 were calculated from those of table 2. Table 3 brings out more clearly than does table 2 the inverse relationship that existed between the percentages of amino nitrogen and basic nitrogen included in the soluble organic nitrogen fraction. In those cases in which the total organic nitrogen differed in plants grown in the different solutions, no significance can be attached to the ratios between the percentages of amino nitrogen and the corresponding percentages of basic nitrogen in the organic fraction. But table 3 clearly shows that, although the amounts of organic nitrogen in the roots of the plants 41 days old grown in the pH 5, pH 6, and pH 7 solutions

TABLE 3

*Organic nitrogenous fractions in roots of plants used for absorption tests*

Organic nitrogen expressed as percentage of tissue on green weight basis; fractions expressed as percentages of total soluble organic nitrogen.

	AGE OF PLANTS					
	41 days				52 days	
	4.0	5.0	6.0	7.0	4.0	7.0
pH of solutions.....	4.0	5.0	6.0	7.0	4.0	7.0
Organic N in tissue.....	0.045	0.060	0.059	0.060	0.060	0.072
Organic N.....	100.0	100.0	100.0	100.0	100.0	100.0
Basic-free amino N.....	43.2	45.2	57.5	62.9	38.5	56.4
Basic N.....	43.9	39.5	34.2	26.0	44.2	26.6
Amide N.....	11.5	11.2	9.3	9.4	9.8	15.4
Other N.....	1.4	4.1	-1.0	1.7	7.5	1.6

and in those 52 days old grown in the pH 4 solution were all practically the same, the percentages of basic nitrogen and of amino nitrogen included in the total soluble organic nitrogen were quite different in plants grown in solutions of different pH. The data show that the percentage of basic nitrogen was relatively low when the percentage of amino nitrogen was high, and vice versa.

This reciprocal relationship was definitely and consistently correlated with the pH of the culture solutions, and, consequently, with the rates of absorption of  $\text{NH}_4\text{-N}$  and of  $\text{NO}_3\text{-N}$ . It is noteworthy that the percentages, as well as the absolute amounts, of basic nitrogen were lowest in the plants 41 days old grown in the pH 7 solution. In these same plants the concentrations of  $\text{NH}_4\text{-N}$  and of amino nitrogen were the highest, and the rate of absorption of  $\text{NO}_3\text{-N}$  was the lowest. This observation suggests that, although  $\text{NH}_4\text{-N}$  may readily pass over into the amino form in the roots of the tomato, it is not readily elaborated there into more complex forms.

Furthermore, the degree of elaboration of the basic nitrogen present in the roots of the plants 41 days old can be correlated with the aforementioned results. A consideration of the method of fractionating the extracts will show that the difference between the total amino nitrogen (not reported in the tables) and the basic-free amino nitrogen gives the alpha-amino nitrogen which was included in the basic precipitate. The results of such calculations showed that in the roots of the plants 41 days old the alpha-amino nitrogen included in the basic fraction comprised a much higher percentage of the basic nitrogen when the plants were grown in the pH 7 solution than when they were grown in the pH 4 solution, and that a gradient was evident in the intermediate solutions. The alpha-amino nitrogen included in the basic precipitates from the extracts of the roots ranged from 24.7 per cent of the basic nitrogen in the plants grown in the pH 4 solution to 65.8 per cent in the plants grown in the pH 7 solution. The same trend, although less pronounced, was evident in the plants 52 days old.

These data indicate that more diamino acids and less complex compounds, having several nitrogen atoms for each alpha-amino radical, must have been present in the basic fraction from the roots of the plants grown in the pH 7 solution than from those grown in the pH 4 solution. That is, not only did the basic nitrogen comprise a smaller fraction of the total soluble organic nitrogen in the roots of the plants grown in the high pH solution than in those grown in the low pH solution, but also the basic nitrogen precipitated was less complex in the former than in the latter. It is recognized, however, that the analytical methods on which these data are based have certain limitations when applied to plant extracts. Plimmer (15) has presented evidence of the inclusion of other forms of nitrogen than the alpha-amino form in the nitrogen obtained by the Van Slyke (22) method, and Vickery (23) has shown that the nitrogen precipitated by phosphotungstic acid from plant extracts is not composed simply of basic amino acids. The concentrations of amide nitrogen not only were low throughout the plants, but also were not influenced by the pH of the culture solution.

#### *Composition of Stems and Leaves*

Although it is possible to trace a relationship between the rates of absorption of  $\text{NH}_4\text{-N}$ , the concentrations of  $\text{NH}_4\text{-N}$ , and, to some degree, the concentrations of amino and basic nitrogen in the tissues of the roots of the plants, the course of assimilation of nitrogen in the tops is not evident. The amounts of nitrogen included in the various nitrogenous fractions, expressed as percentages of the green weight of the tissue, in the stems and petioles are presented in table 4. For brevity, these fractions are referred to as "stems." Similar data from the leaves are given in table 5. Values for protein nitrogen in the stems and leaves, a fraction which was not determined in the roots because of the lack of sufficient material, are included. The average values presented in these tables are based on the same number of plants as are those of table 2.



No graphs are presented to illustrate the relation between rates of absorption of nitrogen and the amounts of inorganic nitrogen in the tissues of the tops because the variation in inorganic nitrogen in the tops with variation in pH of the external medium cannot be closely correlated with absorption by the roots. Instead, the composition of the three different fractions of the plants—roots, stems, and leaves may be compared in an attempt to trace the assimilation of nitrogen throughout the plants.

The data for  $\text{NO}_3\text{-N}$  in tables 2, 4, and 5 reveal that there was not a decreasing concentration of  $\text{NO}_3\text{-N}$  in the plants from roots to leaves. Instead,  $\text{NO}_3\text{-N}$  accumulated in extremely high quantities in the stems as compared with the roots and leaves, a phenomenon probably related to the different amounts of

TABLE 4  
*Nitrogenous fractions in stems of plants used for absorption tests*  
Nitrogen expressed as percentage of tissue on green weight basis

	AGE OF PLANTS					
	41 days				52 days	
	4 0	5 0	6.0	7 0	4 0	7.0
pH of solutions . . . . .						
Average green weight of stems per plant in gm. . . . .	26 2	27 2	30.7	26 8	69 7	61.0
Per cent dry matter . . . . .	5 55	5 23	5 00	4.87	7.17	6 92
Total N . . . . .	0 248	0.244	0 236	0.221	0 310	0 275
Protein N . . . . .	0 037	0.043	0 028	0.022	0 096	0.080
Total soluble N . . . . .	0.211	0 201	0 209	0.199	0 215	0.195
Soluble organic N . . . . .	0.068	0 064	0.068	0 068	0.068	0.080
Basic-free amino N . . . . .	0.024	0.021	0 021	0 020	0.039	0 039
Basic N . . . . .	0 015	0.010	0 012	0.012	0.016	0.016
Amide N . . . . .	0 008	0.007	0.007	0.005	0.014	0.016
Other N . . . . .	0.021	0.025	0.029	0.032	—0.001	0.009
Ammonia N . . . . .	0 016	0.015	0 015	0.015	0.022	0 019
Nitrate N . . . . .	0.128	0 123	0 126	0.115	0.124	0.096

reducase in the different parts of the plant (6). Furthermore, the rather small differences in concentration of  $\text{NO}_3\text{-N}$  in the roots between plants grown in solutions of different pH were paralleled by relatively small differences in the corresponding stems and leaves, with one apparent exception: the low concentration of  $\text{NO}_3\text{-N}$  in the stems of the plants 52 days old grown in the pH 7 solution, an interpretation of which is suggested in the discussion of figure 4.

Since tomato stems normally accumulate considerable quantities of  $\text{NO}_3\text{-N}$  (12, 21) when grown in a culture solution including nitrogen in this form, it is difficult to trace the course of assimilation of  $\text{NO}_3\text{-N}$  in these plants. However, the fact that no greater accumulations of  $\text{NO}_3\text{-N}$  occurred in plants absorbing  $\text{NO}_3\text{-N}$  more rapidly than occurred in others absorbing  $\text{NO}_3\text{-N}$  less rapidly

suggests that the pH range which favored the most rapid rate of absorption of  $\text{NO}_3\text{-N}$  also favored the most rapid assimilation of  $\text{NO}_3\text{-N}$ . The results obtained by Tiedjens and Robbins (21), using  $\text{Ca}(\text{NO}_3)_2$  as a source of nitrogen, were quite similar. They found that tomato plants supplied with only  $\text{NO}_3\text{-N}$  were larger when grown in pH 4, pH 5, or pH 6 solutions than when grown in a pH 7 (or pH 3) solution. Less rapid absorption accompanied by less rapid and less complete assimilation of  $\text{NO}_3\text{-N}$  by plants grown in the pH 7 solution than by those grown in the pH 4, pH 5, and pH 6 solutions would produce the results they obtained. Their data show that stems which were lowest in green weight (those grown in the pH 7 solution) included the highest percentage of

TABLE 5  
*Nitrogenous fractions in leaves of plants used for absorption tests*  
Nitrogen expressed as percentage of tissue on green weight basis

	AGE OF PLANTS					
	41 days				52 days	
	4 0	5 0	6 0	7 0	4 0	7 0
pH of solutions . . . . .	4 0	5 0	6 0	7 0	4 0	7 0
Average green weight of leaves per plant in gm. . . . .	19 4	19 8	22 4	20 0	52 0	39.2
Per cent dry matter . . . . .	11 00	10 63	10 18	10 47	11 68	12 52
Total N . . . . .	0 650	0 627	0 563	0 614	0 621	0 677
Protein N . . . . .	0.533	0 515	0 425	0.495	0 479	0.554
Total soluble N . . . . .	0 117	0.113	0.137	0.119	0 142	0.123
Soluble organic N . . . . .	0 080	0.076	0 099	0 079	0.096	0 091
Basic-free amino N . . . . .	0 027	0 025	0.028	0.028	0 033	0 038
Basic N . . . . .	0 031	0.029	0 042	0.037	0.042	0 033
Amide N . . . . .	0 007	0 007	0 007	0.006	0.011	0.012
Other N . . . . .	0 016	0 015	0 023	0 007	0 010	0.008
Ammonia N . . . . .	0.008	0 007	0 007	0 008	0 009	0 008
Nitrate N . . . . .	0 029	0.029	0 031	0.032	0.037	0.024

$\text{NO}_3\text{-N}$ . The foregoing data have shown that the rate of absorption of  $\text{NO}_3\text{-N}$  by younger plants, at least, decreased as the pH of the medium increased.

The data for  $\text{NH}_4\text{-N}$  in tables 2, 4, and 5 show that the concentration of  $\text{NH}_4\text{-N}$  in the tops was comparatively low and that for plants of a given age there was no significant difference in the content of this fraction between plants grown in solutions of different pH. A slightly greater concentration of  $\text{NH}_4\text{-N}$  was found in the stems of the plants 52 days old than in those 41 days old. It should be mentioned that a much greater accumulation of  $\text{NH}_4\text{-N}$  than was found in these particular plants sometimes occurs in the stems of tomato plants. Comparable plants grown in the pH 4 solution in sand culture until they were 70 days old included about three times as much  $\text{NH}_4\text{-N}$  in their stems as did those reported in table 4. This is another example of a phenomenon reported by Sessions and Shive (20).

In contrast to  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$  did not accumulate in the stems or leaves of the plants in any appreciable excess of the concentration in the roots. It is significant that the large differences in concentration of  $\text{NH}_4\text{-N}$  in the roots between plants grown in solutions of different pH were not reflected in the tops. This indicates rapid assimilation of  $\text{NH}_4\text{-N}$  in the roots, further proof of which is found in the fact that high concentrations of amino nitrogen in the roots accompanied high concentrations of  $\text{NH}_4\text{-N}$  there. Although  $\text{NH}_4\text{-N}$  may also be present in cells as a catabolic product resulting from the breakdown of more complex nitrogenous constituents,  $\text{NH}_4\text{-N}$  from such sources could not have been abundant under the experimental conditions prevailing.

Although  $\text{NH}_4\text{-N}$  did accumulate somewhat in the roots of the plants which were absorbing it most rapidly, the accumulation was not excessive and did not result in any differences in concentration of  $\text{NH}_4\text{-N}$  in the tops of plants grown in solutions of different pH. Therefore, with respect to  $\text{NH}_4\text{-N}$ , also, it can be said that the pH range which favored the most rapid rate of absorption of  $\text{NH}_4\text{-N}$  also favored the most rapid assimilation of  $\text{NH}_4\text{-N}$ . This is in confirmation of the results obtained by Tiedjens and Robbins (21), using  $(\text{NH}_4)_2\text{-SO}_4$  as a source of nitrogen for tomato plants grown in solutions ranging from pH 4 to pH 8. They found that as the pH of the solution increased, the weight of the stems increased. More rapid absorption accompanied by more rapid and more complete assimilation of  $\text{NH}_4\text{-N}$  by plants grown in the solution of high pH than by those grown in solutions of low pH would produce greater growth of plants grown in solutions of high pH. Their data show that the largest plants, those from the solution of high pH, contained the least  $\text{NH}_4\text{-N}$  in the stems. The foregoing data have shown that the rate of absorption of  $\text{NH}_4\text{-N}$  greatly increased as the pH of the solution increased.

In general, the absence of many significant differences between nitrogenous fractions from the tops of plants grown in solutions of different pH is very evident. Such a condition was a natural result of the experimental conditions prevailing, namely: (a) the presence of equal concentrations of  $\text{NH}_4\text{-N}$  and of  $\text{NO}_3\text{-N}$  in the culture solutions in sufficient, but not excessive, quantities; (b) a pH range which was not great enough to produce toxicity or restricted growth in the plants; (c) the relatively long days prevailing during the spring; and (d) continuous aeration and continuous renewal of the solutions. Tiedjens and Robbins (21) also found comparatively small differences between the concentrations of corresponding nitrogenous fractions from the stems of tomato plants grown in different solutions of pH 4, pH 4.8, pH 6, and pH 7 when  $\text{NH}_4\text{NO}_3$  was used as a source of nitrogen, in great contrast with the results obtained when either  $\text{NO}_3\text{-N}$  or  $\text{NH}_4\text{-N}$  was used alone.

As a result of the lack of significant differences in the tops, any discussion concerning the relation of the analytical results to the green weights of the plants would be largely speculation.

However, it might be of interest to trace the course of assimilation of  $\text{NH}_4\text{-N}$  and of  $\text{NO}_3\text{-N}$  further by studying the proportions of soluble organic nitrogen

in the tops as well as in the roots. Accordingly, the total soluble organic nitrogen values in the various organs of the plants grown in the several solutions have been plotted in figure 4. It is evident from this figure that there was a gradient in concentration of soluble organic nitrogen in the tissue from a low percentage in the roots to a high percentage in the leaves of plants in corresponding cultures. With one exception, the concentrations of soluble organic nitrogen in the stems were more nearly constantly proportional to those in the roots than to those in the leaves.

Although the soluble organic nitrogen has been treated in the foregoing discussion as a product resulting from the partial assimilation of inorganic nitrogen, it may also be considered as an intermediate product in the partial dissociation of the more complex proteinaceous nitrogen. The results obtained by

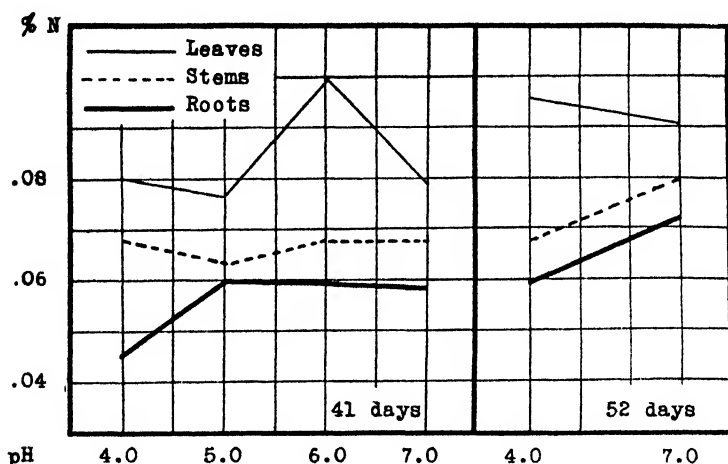


FIG. 4. TOTAL SOLUBLE ORGANIC NITROGEN, EXPRESSED AS PERCENTAGE OF GREEN TISSUE IN LEAVES, STEMS, AND ROOTS OF TOMATO PLANTS

Nightingale, Schermerhorn, and Robbins (12) with tomatoes, after the pioneer work of Kraus and Kraybill (8) have shown that an abundance of soluble organic nitrogen may be present either in weakly vegetative, non-fruitful plants kept in the dark, or in strongly vegetative, non-fruitful plants grown in the light. These workers have shown that the carbohydrate economy of the plants greatly affects the elaboration of protein, growth, and reproduction in plants.

No quantitative analyses for carbohydrates were made in these experiments, but microchemical tests made at the time of the first harvest showed very little starch present in the stems of the plants when 41 days old.

In vegetative plants growing in the light, as were these tomatoes, the end-products resulting from continued absorption and assimilation of nitrogen should be abundant protein nitrogen accompanying a fairly high concentration of soluble organic nitrogen. The data in tables 4 and 5 show the high proportions of protein nitrogen in the leaves as compared with that in the stems, as

well as the relatively large increase in protein nitrogen in the stems as the plants became older. It is evident that the soluble organic nitrogen in the stems of the plants 41 days old exceeded the protein nitrogen in the stems, but that the amount of soluble organic nitrogen in the leaves of the same plants was much less than that of protein nitrogen in the same tissue.

In conclusion, it appears from a consideration of the rates of absorption of  $\text{NH}_4\text{-N}$  and of  $\text{NO}_3\text{-N}$  in relation to the composition of the plants, particularly with respect to the concentrations of  $\text{NH}_4\text{-N}$  and of  $\text{NO}_3\text{-N}$  in the tissues, that each ion was most rapidly assimilated when most rapidly absorbed. This tendency emphasizes the fundamental importance of those properties of protoplasm which determine the rates of absorption of anions and cations from solutions of different hydrogen-ion concentration.

#### SUMMARY

Tomato plants grown in a solution that contained equal concentrations of  $\text{NH}_4\text{-N}$  and of  $\text{NO}_3\text{-N}$  and that was adjusted to pH 4.0, 5.0, 6.0, and 7.0 were first used for absorption tests and were then harvested and analyzed for certain nitrogenous fractions. A summary of the results of the analyses of the plants in relation to rates of absorption of nitrogen is as follows:

The concentration of  $\text{NO}_3\text{-N}$  in the roots of plants of a given age was approximately the same in all of the plants, despite markedly different rates of absorption of  $\text{NO}_3\text{-N}$  from solutions of different pH.

In contrast, the concentration of  $\text{NH}_4\text{-N}$  in the roots varied with the pH of the external medium, higher concentrations of  $\text{NH}_4\text{-N}$  being present in the roots of plants grown in solutions of high pH than in those grown in solutions of low pH. High concentrations of  $\text{NH}_4\text{-N}$  in the roots accompanied high rates of absorption of  $\text{NH}_4\text{-N}$  from the solutions.

High concentrations of  $\text{NH}_4\text{-N}$  in the roots were accompanied by high concentrations of basic-free amino nitrogen, an indication of a rapid formation of amino nitrogen in the roots from the  $\text{NH}_4\text{-N}$  present.

In the roots the percentage of total soluble organic nitrogen composed of basic nitrogen was relatively low whenever the percentage of amino nitrogen in the corresponding extract was high, and vice versa. This relationship was correlated with the pH of the culture solutions in that high concentrations of amino nitrogen were present in the roots of the plants grown in solutions of high pH, from which the rates of absorption of  $\text{NH}_4\text{-N}$  were high.

High proportions of amino nitrogen were included in the basic nitrogen in the roots of those plants which were grown in solutions of high pH and which contained high concentrations of  $\text{NH}_4\text{-N}$  and amino nitrogen.

$\text{NO}_3\text{-N}$  accumulated in extremely high concentrations in the stems as compared with the roots and leaves, but the small variation in concentration of  $\text{NO}_3\text{-N}$  in the roots with variation in pH of the culture medium was paralleled by a relatively small variation in concentration in the stems and leaves.

Since no greater accumulation of  $\text{NO}_3\text{-N}$  in the plants resulted from high rates of absorption of  $\text{NO}_3\text{-N}$  than from low, it is evident that the pH range which favored the most rapid rate of absorption of  $\text{NO}_3\text{-N}$  also favored the most rapid assimilation of  $\text{NO}_3\text{-N}$ .

The concentration of  $\text{NH}_4\text{-N}$  in the tops was comparatively low, and for plants of a given age, there was no significant difference in this respect between plants grown in solutions of different pH, despite the differences found in the roots.

Since no greater accumulation of  $\text{NH}_4\text{-N}$  in the tops of the plants resulted from high rates

of absorption of  $\text{NH}_4\text{-N}$  than from low, and since high concentrations of amino nitrogen in the roots accompanied high concentrations and high rates of absorption of  $\text{NH}_4\text{-N}$ , it is apparent that the pH range which favored the most rapid rate of absorption of  $\text{NH}_4\text{-N}$  also favored the most rapid assimilation of  $\text{NH}_4\text{-N}$ .

As a result of the experimental conditions prevailing, it was not possible to trace in considerable detail the course of assimilation of the two forms of nitrogen in the tops of plants grown in solutions of different pH.

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# A METHOD FOR DETERMINING THE CAPACITY OF A SOIL TO FIX PHOSPHORUS IN DIFFICULTLY AVAILABLE FORM<sup>1</sup>

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It has long been known (1, 3) that when soluble phosphates are applied to soils, they change to less soluble forms, the availability of which varies. If the fixed phosphorus is combined with calcium, it is considered readily available, but if with iron or aluminum, more or less difficultly available.<sup>3</sup> Since soils vary greatly in fixing power, it is important to be able to classify them as to their capacity to fix phosphorus in difficultly available forms. This may be done by treating a moist soil with soluble phosphate, allowing it to stand for a given length of time under moist conditions, and then determining the phosphorus still readily available. Ford (1) has described a laboratory method in which the soil and soluble phosphate are evaporated to dryness at 30°C. This procedure hastens the reaction and greatly reduces the time. Using Ford's method as a basis and starting point, the writer proceeded to perfect the procedure so as to make it shorter and more reliable. The method outlined in this paper is the result of this work.

## EXPERIMENTAL

*The effect of time and temperature.* The work of Scarseth (5), as well as work in this laboratory, and field experience, indicate that time is an important factor in the fixation of phosphates in soils, although much of the fixation in a moist soil takes place almost immediately or within a few hours after the application of a soluble phosphate. After this initial fixation, the change is much slower, and the balance between the readily available and the fixed phosphorus approaches an equilibrium, but with a tendency toward an increase in the fixed phosphorus. Under normal field conditions, equilibrium is practically established in from 1 to 12 months.

<sup>1</sup> Contribution from the department of soils, University of Wisconsin, Madison, Wisconsin. Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

<sup>2</sup> The writer is appreciative of the kindly cooperation of Prof. E. Truog in connection with this study.

<sup>3</sup> The term "fixation" has been used to refer to the change of phosphorus in the soil to forms only slightly soluble in water, and, also, in a more restricted sense, to apply only to the change of phosphorus to the difficultly available form. The latter meaning will be generally understood in this paper.



The effect of time on the fixation of phosphates in the difficultly available form was determined in Miami silt loam and Superior clay from Wisconsin, Memphis silt loam (Mayfield) from Kentucky, and a lateritic soil from the Waipio Substation, Experiment Station Hawaiian Sugar Planters' Association, Hawaii. To each soil was added 400 p.p.m. of phosphorus as monocalcium phosphate dissolved in enough water to make the soil up to optimum moisture. The soils were held at this moisture content and room temperature, and the readily available phosphorus was determined by the Truog method (6, 7) after 2, 10, 20, 40, and 80 days.

It was found that some fixation occurred during the extraction process, and to correct for this error a factor was employed. On the basis of a soil's capacity

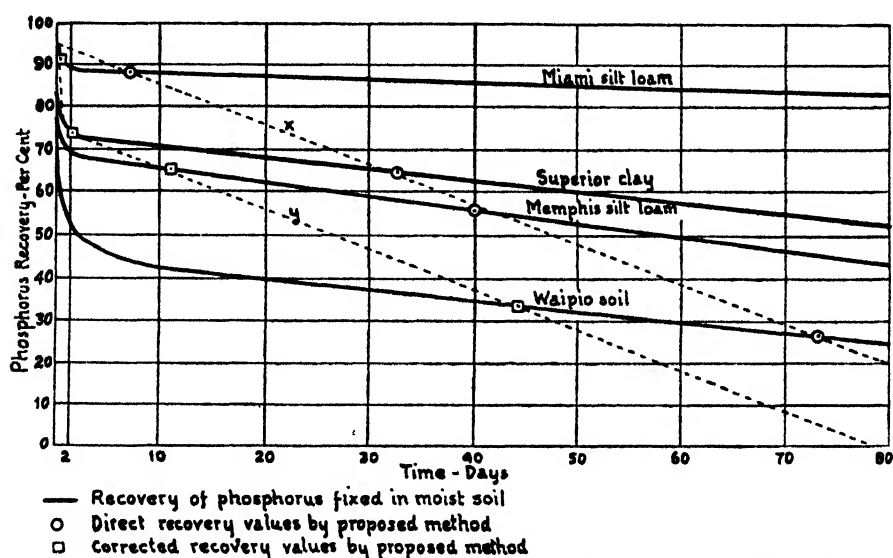


FIG. 1. THE RECOVERY OF APPLIED PHOSPHORUS FIXED IN MOIST SOILS IN READILY AVAILABLE FORM, AND THE RELATION OF THE DIRECT AND CORRECTED RECOVERY VALUES OF THE PROPOSED METHOD TO THESE DATA

to fix 100 per cent of the applied phosphorus in difficultly available form, the factor 1.375<sup>4</sup> was used, and the direct recovery values were multiplied by the proper correction factor. When this correction factor is assumed to follow a straight line, varying from 1.0 at zero fixation to 1.375 at 100 per cent fixation, the increment of recovery is a parabolic function and follows the equation,  $y^2 = 2px$ , in which  $p = 135$ . On this basis the fixation curves for the four soils mentioned were determined and are shown in figure 1.

These curves show that the most pronounced fixation takes place within a

<sup>4</sup> This factor was found experimentally by adding to the soil in the extracting solution 400 p.p.m. of phosphorus as monocalcium phosphate, and then determining the amount of this phosphorus fixed by the soil during a 30-minute period of extraction.

few hours, and that after a transition period, varying from 2 to 10 days, the fixation takes the form of practically a straight line with a tendency to be somewhat asymptotic, and which slowly approaches the zero line of recovery. It was found that heat could, in part, be substituted for time, and values obtained which would show the relative capacities of soils to fix phosphorus in the difficultly available form, and, at the same time, would be fair measures of what would take place under normal field conditions. This was accomplished by refluxing the soil in the phosphate solution at 100°C. for 45 minutes. It was also found that if the soil and the phosphate solution were slowly boiled to dryness, the result was the same as when they were refluxed. From these facts, the following method for determining the capacity of a soil to fix phosphorus in the difficultly available form was developed.

*The method.* One-half gram of 40-mesh soil is placed in a 500-cc. Erlenmeyer flask, and to it are added 2 cc. of a solution of monocalcium phosphate containing 0.1 mgm. of phosphorus per cubic centimeter, and 50 cc. of water. This is an application of 400 p.p.m. of phosphorus. Phosphoric acid or any soluble phosphate may be used, but the monocalcium phosphate is more comparable to the common superphosphate used in the field. The flask is then heated, the contents are boiled gently almost to dryness, and the process is completed on a steam plate or steam bath. The boiling should not be too vigorous, but rapid enough to complete the evaporation in about 45 minutes. In this way superheating is prevented. Into a similar flask is placed a half-gram sample of the untreated soil, and then to each of the two flasks is added 200 cc. of 0.002 *N* sulfuric acid buffered to pH 3. The two are shaken for 30 minutes, filtered, and the phosphorus is determined by the method of Truog and Meyer (6, 7). The amount of phosphorus extracted from the treated soil minus the amount from the untreated soil gives the amount of applied phosphorus recovered. This latter amount, subtracted from the applied phosphorus, gives the phosphorus fixed in difficultly available form. An example of the calculation for Carrington silt loam follows:

Soil + 400 p.p.m. phosphorus gave on extraction,	320 p.p.m. available phosphorus
Soil, untreated, gave on extraction,	20 p.p.m. available phosphorus
Applied phosphorus recovered,	300 p.p.m. available phosphorus
$\frac{300}{400} \times 100 = 75$ per cent, applied phosphorus recovered in readily available form.	

On this basis, 25 per cent of the applied phosphorus was fixed in difficultly available form. If the recovery is to be corrected for the fixation during extraction, the 75 per cent is multiplied by 1.09 (factor for 25 per cent fixation) which results in a corrected recovery value of 81.7 per cent.

The fixation and recovery values by this method for the four soils referred to in figure 1 were determined and are given in table 1. If the direct recovery values from this table are inserted in the proper places in figure 1, the line *x* is established, and if the corrected recovery values are used, the line *y*. The points at which these lines intersect the fixation curves for the different soils

indicate the time at which the fixation of applied soluble phosphorus in a normally moist soil would be equal to the values obtained by the method as outlined. It appears from these data that the direct recovery values and their corresponding fixation values are a little nearer to the fixation values obtained under normal conditions in the field when the length of the growing season is considered than are the corrected values.

TABLE 1

*The fixation in difficultly available form, as measured by laboratory method, and the recovery of soluble phosphate applied to the four soils referred to in figure 1*

SOIL	APPLIED PHOSPHORUS FIXED (DIRECT)	APPLIED PHOSPHORUS RECOVERED IN READILY AVAILABLE FORM	
		Direct	Corrected
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Miami silt loam.....	13.5	86.5	90.8
Superior clay.....	35.5	64.5	73.6
Memphis silt loam (Mayfield).....	44.0	56.0	65.0
Waipio soil (Lateritic).....	74.0	26.0	33.2

TABLE 2

*The pH values, readily available phosphorus, and the phosphorus fixing power of 14 soils*

SOIL	pH VALUE (HYDROGEN ELEC- TRODE)	READILY AVAILABLE NATIVE PHOS- PHORUS	FIXING POWER	
			Dried at 50°C.	Dried at 100°C
		<i>p.p.m.</i>	<i>per cent</i>	<i>per cent</i>
1. Placenta loam (California).....	7.73	72	0.5	11.7
2. Makiki (vir.) (Hawaii).....	7.41	690	45.0	62.5
3. Opauala 6 (Waialua-Hawaii).....	7.11	12	...	60.0
4. Palouse silt loam (Washington).....	6.71	162	0.5	14.3
5. Waipio (Waipio Sub-Sta. Hawaii).....	6.66	88	....	70.8
6. Miami silt loam (Wisconsin)*.....	6.65	74	0.0	8.5
7. Kawaihapai 3 (Waialua-Hawaii)....	6.49	420	10.0	30.0
8. Opauala 18 (Waialua-Hawaii).....	6.09	Trace	67.0	80.0
9. Colby silt loam (Wisconsin).....	6.08	26	35.3	46.5
10. Helemano 2A (Waialua-Hawaii).....	5.94	20	51.0	70.0
11. Carrington silt loam (Wisconsin)....	5.67	20	20.0	25.0
12. Aiken clay adobe (California).....	5.57	Trace	71.0	87.0
13. Manoa (vir.) (Manoa Sub-Sta. Hawaii).....	5.40	124	90.5	100.0
14. A.H.P.C. 631 (Maui-Hawaii).....	4.40	12	....	94.0

\* Not the same Miami referred to in table 1.

Results by this method were also compared with those by a method similar to the one used by Ford (1) in which the same amount of soil and the same amounts of phosphorus and water as used in this method were placed in beakers and held at 50°C. until dry. This required about 24 hours. After this, the

samples were extracted as in the method described. Figures by both methods, together with the pH values and the readily available phosphorus contents obtained with a number of soils, are given in table 2.

#### DISCUSSION

The method of extraction after fixation is essentially that of Truog (6), except that a ratio of soil to solution of 1 to 400 was used. This higher ratio of solvent aids materially in maintaining the pH of the extracting solution at more nearly its original value of 3, and, unless the soil contains appreciable amounts of calcium carbonate, this can be done with only a slight rise. Since the fixation in highly calcareous soils will be generally in the form of calcium phosphate (3), a form which is readily available, this method may be used to best advantage with soils containing not more than a small amount of calcium carbonate. None of the soils listed in table 2 contained appreciable amounts of calcium carbonate.

The figures in table 2 indicate that the amounts of applied phosphorus changed to the difficultly available form may vary from practically nothing to all of the phosphorus applied. This will hold true only up to a certain amount of applied phosphorus. Using 1 per cent citric acid as the extracting solution, McGeorge (4) found that above a certain amount of applied phosphorus there was a dropping off of the percentage fixed, although there was a steady increase in the total amount of phosphorus fixed. Four hundred parts per million does not approach this amount and seems to be a very convenient and satisfactory amount to use.

The higher temperature of evaporation gives from 8 to 18 per cent higher fixation values than the lower temperature, and indicates some fixation with all soils, which seems logical, because a soil like the Miami silt loam should contain some material capable of fixing phosphorus in difficultly available form. Again, some soils should be capable of fixing all of the 400 p.p.m. of applied phosphorus, but none of the soils show this at the lower temperature. On the whole, the higher temperature gives results more nearly in accord with the fixation curves in figure 1, and with what might be expected from longer contact under field conditions. At the same time this method is more convenient because of the shorter time involved.

Of the soils listed in table 2, the laterites from Hawaii and the Aiken clay adobe from California have the greatest capacity for fixing phosphorus in the difficultly available form, apparently, because of the large amount of hydrated ferric oxide which they contain. The Miami silt loam used is typical of a low fixing soil. It not only contains very little active iron or aluminum, but at the same time its pH value is high enough so that it is well supplied with exchangeable calcium, thus holding the applied phosphorus as calcium phosphate.

A method involving water extraction instead of that by a weak acid has been recently proposed by Hance (2), but obviously such a method cannot be used for the estimation of the capacity of a soil to fix phosphorus in the difficultly

available form. Most soils tend to change soluble phosphate to a form relatively insoluble in water, but this may be the readily available calcium phosphate. Basic soils with little or no active iron or aluminum will tend to fix practically all of the applied soluble phosphate in this form.

In some virgin soils containing a relatively large amount of phosphorus associated with organic matter, there is a tendency for the boiling to bring about hydrolysis of a part of this phosphorus and allow it to be fixed in the difficultly available form. However, because of the tendency of each soil to establish its own equilibrium between the readily available and fixed phosphorus, there is a tendency for any phosphorus fixed from this organic source to be balanced by an equal amount of applied phosphorus left readily available. For this reason perhaps, no soil has been found to give an apparent fixation of more than 100 per cent of the applied phosphorus. In most cultivated soils any error from this source has been found to be negligible.

The outlines of this method were furnished A. R. Midgley of the Vermont Experiment Station and were used by Weiser (8) of that station in a very recent work in which he reports obtaining very satisfactory results with Vermont soils.

#### SUMMARY

A method is outlined in which 0.5 gm. of soil is treated with 0.2 mgm. (400 p.p.m.) of phosphorus as monocalcium phosphate in solution in 50 cc. of water, and the suspension boiled to dryness. The readily available phosphorus is then determined by means of 0.002 *N* sulfuric acid buffered to pH 3. This amount, subtracted from the sum of what was added and present in the soil before treatment, gives the amount fixed, which makes it possible to calculate the capacity of the soil to fix phosphorus in difficultly available form.

Results by this method compare favorably with results obtained under field conditions. The time required in the field for a soil to attain the fixation percentage indicated by this method becomes proportionately greater as the capacity of the soil for fixation increases.

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## A COMPARISON OF SUPERPHOSPHATE AND RAW ROCK PHOSPHATE ON DIFFERENT TYPES OF SOIL<sup>1</sup>

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The loam and sandy loam soils of New Jersey are generally fairly constant in phosphoric acid content. In most cases the loams contain about 0.10 to 0.14 per cent phosphoric acid. Eleven samples representing different types of loam soils from the Belvidere area average 0.132 per cent phosphoric acid (2). There are, however, some exceptions to this general statement. Colts Neck loam contains an exceptionally high percentage, ranging from about 0.7 to more than 1 per cent phosphoric acid. Three samples of this soil from Monmouth County averaged 0.94 per cent, with the subsoil containing as much as, and sometimes more than, the topsoil (1). Sassafras and Portsmouth loams may contain as much as 0.20 per cent phosphoric acid. Loams which are derived from limestone formations are likely to contain a slightly higher percentage than are those derived from igneous rocks, shales, and slates.

During the past 25 years this question of the comparative availability of superphosphate and rock phosphate has been carefully studied by a number of the experiment stations in this country and abroad. In 1918, Waggaman and Wagner (12) made a careful analysis of the experimental work with ground raw rock phosphate as a fertilizer. Among others who have made contributions to the subject may be mentioned Brooks (4), Hartwell and Damon (6), McGeorge (8), Mooers (9), Truog (11), Williams, C. B. (16), and Wiancko and Conner (15).

With the exception of the work done at the Hawaiian experiment station, none of these workers has compared the two phosphates on a soil which contains as high a percentage of phosphoric acid as the Colts Neck loam. The question naturally arose, therefore, as to whether crops grown on Colts Neck loam and on other soils which contain an unusual amount of native phosphates would respond to treatments of soluble phosphates or ground rock phosphate. For the purpose of studying this problem three types of soil were obtained for experimental work in cylinders<sup>2</sup> located at the experiment station. The three soils were a Colts Neck loam from Monmouth County, and Sassafras and Portsmouth loams from Middlesex County. The Colts Neck loam was from

<sup>1</sup> Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

<sup>2</sup> The type of cylinder that has been used at this station for the past 35 years.

good land where clover was growing and was only slightly acid, pH 6.5, with a lime requirement (CaO by Veitch method) of 100 pounds to the acre. It contained 0.77 per cent total phosphoric acid. The Sassafras loam was taken from an abandoned field in the vicinity of Dayton and was poorer than the average soil of this type. It was distinctly acid, having a pH of 6.2 and a lime requirement of 600 pounds calcium oxide to the acre. It contained 0.1 per cent total phosphoric acid. The Portsmouth loam was taken from a swampy area in the vicinity of Dayton and had not been under cultivation. It was strongly acid, having a pH of 5.2 and a lime requirement of 4,000 pounds calcium oxide to the acre. It contained 0.2 per cent total phosphoric acid. A partial chemical analysis of these soils is herewith given (3).

	Colts Neck loam per cent	Sassafras loam per cent	Portsmouth loam per cent
N.....	0.126	0.053	0.451
P <sub>2</sub> O <sub>5</sub> .....	0.772	0.108	0.202
K <sub>2</sub> O.....	1.660	0.950	1.341
CaO.....	0.660		
MgO.....	0.750		
Fe <sub>2</sub> O <sub>3</sub> .....	25.180		
Al <sub>2</sub> O <sub>3</sub> .....	5.840		
Organic carbon.....		1.380	5.710
pH.....	6.5	6.2	5.2
Lime requirement (Veitch).....	100	600	4000

#### PLAN OF TREATMENT

The plan of treatment provides for applications of superphosphate to give 0.5, 1.0, 2.5, and 5.0 gm. of phosphoric acid per cylinder on one section and equivalent amounts of ground phosphate rock (ground as for the preparation of superphosphate) on another section. The superphosphate treatments are equivalent to 100, 200, 500, and 1,000 pounds to the acre. In addition to the phosphate, all cylinders received 10 gm. nitrate of soda, or the equivalent in nitrate of soda and sulfate of ammonia, and 10 gm. of muriate of potash, these amounts being equivalent to 320 pounds of nitrate of soda and 320 pounds of muriate of potash to the acre. All fertilizers were applied annually. To one-half of the cylinders that received the ground phosphate rock, calcium sulfate was applied in amounts equivalent to the amount of calcium sulfate contained in the superphosphate used on the other section.

A limed and an unlimed series was provided for each type of soil.

The soils were collected and placed in the cylinders in the fall of 1922. Fertilizers and lime were applied in accordance with the plan outlined in the foregoing, and rye was planted on the Sassafras loam and the Portsmouth loam. The Colts Neck loam was placed in the cylinders too late for the planting of rye.

*Colts Neck Loam*

*Limed section.*<sup>3</sup> Barley and soybeans were grown on this soil in 1923; potatoes in 1924, 1925, 1926, and 1927; and grain and forage crops in 1928 to 1932 inclusive. [The results for the potatoes have been published (3).] Crop yields for the years 1923, 1927, 1928, 1929, 1930, 1931, and 1932 for the limed section of the Colts Neck loam are shown in table 1. It will be noted that two crops were grown in 1923, 1927, and 1930.

TABLE 1

*Yields of dry matter in a comparison of superphosphate and raw rock phosphate on Colts Neck loam—limed section—1923-1932*

SPECIAL TREATMENT	BARLEY 1923		SOYBEAN HAY 1923	BUCKWHEAT 1927	CORN FORAGE 1928	OATS 1929		RAPE 1930	SOYBEAN HAY 1930	BARLEY 1931		BARLEY 1932		ANNUAL AVERAGE		
	Grain	Straw				Grain	Straw			Grain	Straw	Grain	Straw	Grain	Straw	Hay, etc.
<i>gm per cylinder</i>	<i>gm</i>	<i>gm</i>	<i>gm</i>	<i>gm</i>	<i>gm</i>	<i>gm</i>	<i>gm</i>	<i>gm</i>	<i>gm</i>	<i>gm</i>	<i>gm</i>	<i>gm</i>	<i>gm</i>	<i>gm</i>	<i>gm</i>	<i>gm</i>
<i>Superphosphate Section</i>																
Check . . . . .	47	60	82	28	343	53	96	90	116	38	68	23	46	40	68	165
0.5 gm. P <sub>2</sub> O <sub>5</sub> * . . . . .	57	65	93	52	360	51	97	100	134	43	76	20	40	43	70	185
1.0 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	62	68	98	58	423	59	98	101	147	47	90	37	63	51	80	207
2.5 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	62	71	92	57	412	50	108	114	150	52	94	32	66	49	85	206
5.0 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	63	70	85	60	437	57	102	105	144	55	96	46	87	55	89	208
Average of 4 treatments	61	69	92	57	408	54	101	105	144	49	89	34	64	50	81	202
<i>Raw Rock Phosphate Section</i>																
Check . . . . .	57	64	87	51	413	60	92	90	138	47	78	46	74	53	77	195
0.5 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	54	64	79	52	364	56	97	90	116	47	85	39	71	49	79	175
1.0 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	56	65	77	46	360	56	93	87	101	45	84	48	75	51	79	168
2.5 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	55	66	68	40	353	54	91	102	113	38	80	43	74	48	78	169
5.0 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	59	63	80	51	377	51	107	102	102	43	87	45	73	50	83	178
Average of 4 treatments	56	65	76	47	364	54	97	95	108	43	84	44	73	50	80	173

\* 0.5 gm. per cylinder is equal to 100 pounds superphosphate per acre.

The averages in table 1 show that the superphosphate treatment gave some increase over the check. For example, the average increase of hay and forage with 0.5 gm. P<sub>2</sub>O<sub>5</sub> (100 pounds superphosphate to the acre) is 20 gm., which is equivalent to 640 pounds to the acre. For the hay and forage the increase with 200 pounds of superphosphate to the acre is about twice as much as with 100 pounds. The 500 and 1,000-pound applications did not give much increase over the 200-pound application.

<sup>3</sup> In July, 1923, the pH of the limed soil was 7.1 and of the unlimed soil 6.65; the corresponding figures for July, 1932, were 7.17 and 6.18.



For the ground rock section the phosphate treatments show little increase over the check. Indeed, in a number of cases the yields with the phosphate rock are somewhat less than the yields on the check cylinders. This would indicate that the phosphorus of the ground rock is no more available than the natural phosphate of the soil. Such increases as are noted occur with the soybean hay and corn forage rather than with the grain. The average yields for this section are in most cases slightly less than the averages for the superphosphate section.

TABLE 2

*Yields of dry matter in a comparison of superphosphate and raw rock phosphate on Colts Neck loam—unlimed section—1923-1932*

SPECIAL TREATMENT	BARLEY 1923		SOYBEAN HAY 1923	BUCKWHEAT 1927	CORN FORAGE 1928	OATS 1929		RAPE 1930	SOYBEAN HAY 1930	BARLEY 1931		GRAIN and STRAW 1932	ANNUAL AVERAGE		
	Grain	Straw				Grain	Straw			Grain	Straw		Grain	Straw	Hay, etc.
gm. per cylinder	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
<i>Superphosphate Section</i>															
Check . . . . .	57	61	72	50	345	57	88	79	97	39	65	61†	51	71	141
0.5 gm. P <sub>2</sub> O <sub>5</sub> * . . . . .	55	63	74	59	368	52	89	87	108	44	73	80†	50	75	155
1.0 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	56	72	84	44	419	52	87	69	119	44	77	71	51	79	161
2.0 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	61	73	79	51	417	50	92	103	131	39	74	81†	50	80	172
5.0 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	62	73	70	47	430	58	88	111	113	46	89	101	55	83	174
Average of 4 treatments	59	70	77	50	409	53	89	93	118	43	78	83	52	79	166
<i>Rock Phosphate Section</i>															
Check . . . . .	54	64	71	54	381	46	95	72	99	42	84	83	47	81	152
0.5 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	60	69	76	50	341	47	93	88	110	49	82	75	52	81	148
1.0 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	55	68	65	45	360	52	87	98	93	42	77	109†	50	77	154
2.5 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	56	65	67	42	340	52	89	101	105	46	83	99	51	79	151
5.0 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	46	62	57	54	319	54	90	93	92	40	87	108	47	80	145
Average of 4 treatments . . . . .	54	66	66	48	340	51	90	95	100	44	82	98	50	79	150

\* 0.5 gm. per cylinder is equal to 100 pounds superphosphate per acre.

† One cylinder only.

*Unlimed section.* The dry weights for the unlimed section of the Colts Neck loam are given in table 2, which shows that the yields on this section were somewhat lower than the yields on the limed section, notwithstanding the fact that the soil was only moderately acid.

In a number of instances superphosphate has given some increase in yield of dry matter over the check cylinders, but in most cases this increase would not justify the application of 200 or even 100 pounds of superphosphate to the

acre. It is probable that only in the case of the corn forage would the increase justify the use of 500 pounds to the acre.

For the rock phosphate section the check cylinders frequently gave yields equal to, or greater than, the yields on the treated cylinders. The averages for this section do not indicate any value for the rock phosphate, either in small or large amounts.

TABLE 3

*Yields of dry matter in a comparison of superphosphate and raw rock phosphate on Sassafras loam—limed section—1923-1932*

SPECIAL TREATMENT	RYE 1923		SOYBEAN HAY 1923		CORN FORAGE 1928		OATS 1929		RYE 1930		SOYBEAN HAY 1930		BARLEY 1931		BARLEY 1932		ANNUAL AVERAGE		
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Hay, etc
<i>gm. per cylinder</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
<i>Superphosphate Section</i>																			
Check . . . . .	25	63	74	399	97	49	130	30	62	36	52	30	59	187					
0.5 gm. P <sub>2</sub> O <sub>5</sub> * . . . . .	29	82	60	417	103	60	127	35	69	20	40	28	64	192					
1.0 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	28	76	62	433	102	59	128	31	78	29	57	29	71	196					
2.5 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	24	76	58	509	106	61	143	33	89	34	71	30	79	219					
5.0 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	24	75	66	507	105	66	146	37	93	37	79	33	82	222					
Average of 4 treatments . .	26	77	62	467	104	62	136	34	82	30	62	30	74	207					
<i>Raw Rock Phosphate Section</i>																			
Check . . . . .	20	65	68	386	91	56	130	35	70	33	65	30	67	183					
0.5 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	23	67	71	375	103	56	135	37	73	35	62	32	67	185					
1.0 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	24	66	74	413	103	46	138	34	67	33	54	30	63	194					
2.5 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	20	64	65	377	113	53	129	32	69	31	40	28	58	184					
5.0 gm. P <sub>2</sub> O <sub>5</sub> . . . . .	19	67	70	352	96	45	127	33	67	29	51	27	62	172					
Average of 4 treatments . .	22	66	70	379	104	50	132	34	69	32	52	29	63	184					

\* 0.5 gm. per cylinder is equal to 100 pounds superphosphate per acre.

#### SASSAFRAS LOAM

*Limed section.* The fertilizer and lime treatments and the crops grown were the same for this soil as for the Colts Neck loam. The yields for the limed section are shown in table 3. A study of the averages for the superphosphate section shows, in most cases, only slight increases for the phosphate treatments as compared with the check. Generally, the increases in hay, straw, and forage were greater than the increases in grain. The average increase in hay and forage for the 500-pound application lacked only 3 gm. per cylinder of being as much as the increase with the 1,000-pound application.

The yields for the ground rock section are generally slightly less than for the superphosphate section, and they likewise show a smaller increase over the check. Indeed, in the majority of cases the yields are practically as high for the check cylinders as for those that received the heaviest phosphoric acid treatment. It appears, therefore, that even on this soil, where a deficiency of available phosphoric acid would be expected, there was very little response to the phosphate treatment.

*Unlimed section.* The yields of rye and soybeans in 1923 and of corn forage in 1928 on this soil were very nearly as good as corresponding yields on the

TABLE 4

*Yields of dry matter in a comparison of superphosphate and raw rock phosphate on Portsmouth loam—limed section—1923, 1930, 1931, 1932*

SPECIAL TREATMENT	RYE 1923		SOYBEAN HAY 1923	RYE 1930	SOYBEAN HAY 1930	BARLEY 1931		BARLEY 1932		ANNUAL AVERAGE		
	Grain	Straw				Grain	Straw	Grain	Straw	Grain	Straw	Hay, etc.
<i>gm. per cylinder</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
<i>Superphosphate Section</i>												
Check . . . . .	36	90	78	114	140	30	62	28	53	31	68	166
0.5 gm. $P_2O_5$ *. . . . .	50	121	75	104	151	41	76	29	50	40	82	165
1.0 gm. $P_2O_5$ . . . . .	32	84	87	93	163	39	78	32	50	34	71	172
2.5 gm. $P_2O_5$ . . . . .	29	88	84	100	160	42	86	38	66	36	80	172
5.0 gm. $P_2O_5$ . . . . .	33	102	89	116	146	43	90	53	65	43	86	175
Average of 4 treatments. . .	36	99	84	103	155	41	83	38	58	38	80	171
<i>Raw Rock Phosphate Section</i>												
Check . . . . .	36	99	81	110	150	38	74	37	53	37	75	171
0.5 gm. $P_2O_5$ . . . . .	27	83	87	90	143	39	71	15	38	27	64	160
1.0 gm. $P_2O_5$ . . . . .	27	88	78	85	153	39	75	42	55	36	73	158
2.5 gm. $P_2O_5$ . . . . .	42	109	78	90	167	41	88	38	55	40	84	168
5.0 gm. $P_2O_5$ . . . . .	35	105	81	65	161	44	87	46	64	42	85	154
Average of 4 treatments. . .	33	96	81	83	156	41	80	35	53	36	77	160

\* 0.5 gm. per cylinder is equal to 100 pounds superphosphate per acre.

limed section, but except for the corn forage neither the superphosphate nor the rock phosphate had much effect on the yield of dry matter. By 1929 the soil had become so acid that yields were very much reduced. The rye and soybeans in 1930 were a failure, the barley crop in 1931 was scarcely worth harvesting, and no crop was planted in 1932. By this time the soil was so acid—pH 5.0 to 5.3—that even the 1,000-pound application of phosphate had little effect.

The yields of dry matter from this section are so irregular that their inclusion in the paper is not justified.

## PORTSMOUTH LOAM

*Limed section.* The plan of treatment was the same as for the Colts Neck and Sassafras soils. However, potatoes were grown on this soil for 2 years (1928 and 1929) after the growing of potatoes had been discontinued on the other two soils. The yields were irregular and are not recorded here.

The dry weights of the other crops for the limed section are shown in table 4. An examination of the table brings out the fact that there is very little difference between the yields from the check plots and those from the phosphate treated plots. There is also little difference between the yields from the superphosphate plots and the rock phosphate plots. Thus it would appear that grain crops and soybeans on Portsmouth loam, with a high percentage of organic matter and phosphoric acid, do not respond to phosphate treatment.

*Unlimed section.* The yields on the unlimed section of this soil were very irregular and decreased rapidly as the soil became more acid, and therefore have no value for publication. The pH of the soil in May, 1932, when no crop was growing, varied between 4.9 and 5.3.

## GENERAL DISCUSSION

On well-limed Colts Neck loam soil, which contains an exceptionally high percentage of phosphoric acid, superphosphate gave some increase over the check when used for general farm crops, but in a number of cases the application of 200 pounds to the acre or even 100 pounds of the superphosphate did not give a *profitable* increase.

If one may judge by the yields of dry matter, the rock phosphate, which was applied in amounts equivalent to the superphosphate, was little, if any, more available than the native soil phosphates.

Neither of the phosphates was definitely effective on the unlimed section of this soil.

On the limed Sassafras loam soil with a percentage of phosphoric acid about normal, most of the crops showed some response to the superphosphate treatment, but practically no response to the rock phosphate.

The soil of the unlimed series of the Sassafras loam was too acid to grow good crops, either with or without phosphate treatment.

Applications of superphosphate on limed Portsmouth loam, which also contains a rather high percentage of phosphoric acid, gave very little increase over the check. The yields on the unlimed series were so irregular as to make the data of little value.

In this connection it may be pointed out that 20 years ago Whitson and Weir (14) called attention to the fact "that soils which are acid are low in available phosphorus and will respond to an application of phosphate fertilizers."

Fudge (5) studied samples of soil from the nitrogen availability plots of the New Jersey Agricultural Experiment Station and reported that lime increased phosphate solubility on all plots except 13B. He found much more available phosphoric acid in the soil from the limed section of these plots than from the unlimed section. In further commenting on his studies of these soils he says:

Although the normal phosphates of iron and aluminum, as such, are relatively available to plants, they are very easily hydrolyzed, forming relatively unavailable basic phosphates of iron and aluminum. When lime is applied to such soils, however, the acidity is greatly decreased, and the calcium of the lime replaces much of the hydrogen of the exchange complex and furnishes calcium for the formation of the relatively available calcium phosphate.

Lyon and Buckman (7), discussing the use of soluble phosphates, say:

When the soil is acid and, therefore, lacking in effective calcium, the phosphates react with the active aluminum and iron, forming very insoluble and unavailable calcium-iron-aluminum phosphates. For this reason plenty of calcium should be present in the soil when superphosphate is used in order to insure the full effectiveness of the fertilizer. Because of the precipitation of the active aluminum, superphosphate seems to alleviate the physiological influence of soil acidity, although it may tend to increase the hydrogen ion concentration of the soil. Since lime is cheaper and more effective, it is better to reduce the acidity of the soil with this material and utilize the superphosphate as a fertilizer.

Wheeler (13) points out that liming increases the efficiency of superphosphate but decreases the efficiency of raw rock.

#### SUMMARY

Three types of soil—Colts Neck loam, Sassafras loam, and Portsmouth loam—were used in comparing superphosphate and rock phosphate for growing potatoes, grain, corn forage, and soybeans.

The work was carried out in cylinders of the type that has long been in use at this station.

Complete fertilizer was used in all cases except in the check cylinders, where phosphoric acid was entirely omitted.

Superphosphate was used at rates to give 100, 200, 500, and 1,000 pounds to the acre, and rock phosphate in equivalent amounts.

A series with lime and without lime was provided for each type of soil and for both phosphate treatments.

On well-limed Colts Neck loam, which is unusually rich in total phosphoric acid, grain, corn forage, and soybean hay did not, in the majority of cases, show a pronounced response to applications of superphosphate. The response was greater in the case of soybean hay and corn forage than for grain and straw. Superphosphate at the rate of 200 pounds an acre gave very nearly as great increase as 500 and 1,000 pounds.

On the rock phosphate section yields were frequently larger without phosphate treatment than with it. The averages for the grain and hay show this. This section, on the average, gave about as much grain and straw as the superphosphate section but less hay and corn forage.

On the unlimed section the yields with both superphosphate and rock phosphate are very little more than the yields on the check cylinders.

As a whole, the unlimed section gave somewhat lower yields than the limed section.

On Sassafras loam most of the crops gave a slight response to superphosphate

on the limed section. On this section they gave little or no response to the rock phosphate. On the unlimed section of this soil there was little response to either the superphosphate or rock phosphate, and by 1929 the soil had become so acid that the crops were of little value. After 1931 the unlimed section was discontinued.

The crops on the limed section of the Portsmouth loam gave but little response to the phosphate treatments.

Yields were so irregular on the unlimed section that they are not of value for record.

With conditions similar to those under which this work was carried out, it would seem to be a waste of money to use any form of phosphate on strongly acid soils or on soils such as Colts Neck loam and Portsmouth loam which are naturally well supplied with phosphorus.

Lime applied to such soils increases the availability of the natural phosphates and also the effectiveness of applied fertilizers.

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## AUTHOR INDEX

- Allison, F. E.**, and Ludwig, C. A. Cause of decreased nodule formation on legumes supplied with abundant combined nitrogen, 431-443.
- Anand, B.** *See* Puri, A. N.
- Anderson, D. A.** *See* Walker, R. H., and Brown, P. E.
- Asghar, A. G.** *See* Puri, A. N., Taylor, E. M., and.
- Auer, V.** *See* Dachnowski-Stokes, A. P.
- Black, I. A.** *See* Walkley, A.
- Blair, A. W.**, Prince, A. L., and Winterberg, S. H. A comparison of superphosphate and raw rock phosphate on different types of soil, 483-491.
- Brown, L. A.** Oxidation reduction potentials in soils: I. Principles and electrometric determination, 65-76.
- Brown, P. E.** *See* Walker, R. H., Anderson, D. A., and.
- Byers, H. G.** *See* Middleton, H. E.
- Camargo, T. de**, and Mello, P. C. de. Influence of eight years' successive fertilizing with concentrated potash salts and with kainit on the replaceable bases of the "Terra Roxa" soil, 167-178.
- Carroll, W. R.** A study of *Rhizobium* species in relation to nodule formation on the roots of Florida legumes: I, 117-135; II, 227-241.
- Clark, H. E.**, and Shive, J. W. Influence of the pH of a culture solution on the rates of absorption of ammonium and nitrate nitrogen by the tomato plant, 203-225; influence of the pH of a culture solution on the assimilation of ammonium and nitrate nitrogen by the tomato plant, 459-476.
- Conrad, J. P.** Relation of colloid dispersion in soils to chemical changes induced by biological transformations of organic materials, 179-201.
- Corbet, A. S.** Studies on tropical soil microbiology: I. The evolution of carbon dioxide from the soil and the bacterial growth curve, 109-115.
- Dachnowski-Stokes, A. P.**, and Auer, V. American Peat Deposits (book review), 77.
- Davidson, O. W.**, and Shive, J. W. Influence of the hydrogen-ion concentration of the culture solution upon the absorption and assimilation of nitrate and ammonium nitrogen by peach trees grown in sand cultures, 357-385.
- Dean, L. A.** Electrodialysis as a means of studying the nature of soil phosphates, 253-266.
- Hance, F. E.** Plant feeding by Briquette fertilization, 445-457.
- Heck, A. F.** Phosphate fixation and penetration in soils, 343-355; a method for determining the capacity of a soil to fix phosphorus in difficultly available form, 477-482.
- Holben, F. J.** *See* White, J. W., and Jeffries, C. D.
- Isaak, P.** Nature of slick soil in southern Idaho, 157-165.
- Jeffries, C. D.** *See* White, J. W., Holben, F. J., and.
- Krauss, B. H.** *See* Sideris, C. P.
- Ludwig, C. A.** *See* Allison, F. E.
- Lundblad, K.** Studies on podzols and brown forest soils: I, 137-155.
- Lundegårdh, H.** Sven Odén (obituary), 429.
- MacIntire, W. H.**, Shaw, W. M., and Robinson, B. Distinction between magnesium absorbed and that exchangeable, four years after lysimeter incorporations of oxides and carbonates, 289-303.
- Magistad, O. C.** Relation between replaceable potassium and field response to potash in Hawaiian soils, 99-103.
- Mello, P. C. de.** *See* Camargo, T. de.
- Middleton, H. E.**, and Byers, H. G. Settling volume of soils, 15-27.
- Moyer, T. R.** Calcium cyanamid as a nitrogenous fertilizer, 305-331.
- Piland, J. R.** *See* Willis, L. G.
- Powers, W. L.** Preservation of soils against degeneration, 333-341.



- Pugh, A. J.** Laws of soil colloidal behavior: XIV. Aging of colloids and base exchange, 403-427.
- Puri, A. N.** A new method of determining base exchange capacity of soils, 105-108.
- Puri, A. N., and Anand, B.** An electro-metric-titration method of finding the pH value and lime requirement of soils, 49-58.
- Puri, A. N., Taylor, E. M., and Asghar, A. G.** A new apparatus for measuring soil shrinkage, 59-63.
- Robinson, B.** See **MacIntire, W. H., Shaw, W. M., and**
- Salgado, M. L. M.** A critical examination of analytical methods used in the determination of exchangeable potassium and sodium of soils, 39-48.
- Shaw, W. M.** See **MacIntire, W. H., and Robinson, B.**
- Shive, J. W.** See **Clark, H. E.; Davidson, O. W.**
- Sideris, C. P., and Krauss, B. H.** Effect of sulfur and phosphorus on the availability of iron to pineapple and maize plants, 85-97.
- Taylor, E. M.** See **Puri, A. N., and Asghar, A. G.**
- Volk, N. J.** Fixation of potash in difficultly available form in soils, 267-287.
- Walker, R. H., Anderson, D. A., and Brown, P. F.** Physiological studies on Rhizobium: I. The effect of nitrogen source on oxygen consumption by Rhizobium leguminosarum Frank, 387-401.
- Walkley, A., and Black, I. A.** An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method, 29-38.
- Wheeting, L. C.** A migration method for the determination of replaceable bases in soils, 243-252.
- White, J. W., Holben, F. J., and Jeffries, C. D.** Influence of soil acidity upon the decomposition of organic matter in soils, 1-14.
- Willis, L. G., and Piland, J. R.** Influence of copper sulfate on iron absorption by corn plants, 79-83.

## SUBJECT INDEX

- Acid phosphate versus rock phosphate on different soil types, 483-491
- Acidity, *see* Soil acidity.
- Alfalfa, effect of nitrates on, 434.
- Alkali soils, changes in, due to reclamation, 338.
- Aluminum—
  - effect on phosphate fixation, 344.
  - hydroxide sol, preparation of, 404.
  - silicate, aging of, and its effect on absorption of barium, 409.
- Ammonia nitrogen—
  - in plants, method of determining, 461
  - for plants, 203, 357, 459.
- Bacteria—*see also* Microorganisms.
  - cowpea and soybean, efficiency of, on cowpea plants, 228.
  - growth curve of, and carbon dioxide evolution, 109.
  - number of, in soils variously treated, 11
- Base—
  - exchange—
    - iron and aluminum in, 420.
    - phenomenon of, a discussion, 415.
    - relation to aging of colloids, 403.
  - exchange capacity—*see also* Cation.
    - lime effects on, 335.
    - magnesium absorption and, 300.
    - method of determining, 105-108.
    - of silicates of iron, 423.
    - of soils or soil colloids after certain treatments, 144.
- Bases—
  - adsorbed, and state of saturation, 174
  - replaceable—
    - in slick soils, 160.
    - in "terra roxa" of Brazil, 167.
    - methods of determining, review, 243.
    - migration method for the determination of, 243.
    - relation to rainfall, 86.
- Buffer capacity of "terra roxa," 170.
- Calcium—
  - cyanamid as a nitrogenous fertilizer, 305-331.
  - effect on phosphate fixation, 344.
- Carbon—
  - dioxide—
    - evolution of, from the soil and the bacterial growth curve, 109.
    - production of, in relation to treatment, 5-11.
    - methods of determining, 32.
- Cation exchange reactions and biochemical reactions, 192; *see also* Base exchange.
- Cellulose—
  - decomposition as affected by lime, 12.
  - effect on carbon dioxide production in soils, 5, 6, 10.
- Colloids—*see also* Soil colloids, Soils colloid content.
  - aging of, and base exchange, 403-427
  - content of, in certain soils, 16.
  - dispersion of—
    - coefficient determination on, 161.
    - in soils induced by biological activities, 179.
  - exchange capacity of soil, 144
  - settling volume of, 22.
- Copper sulfate, influence of, on absorption of iron by corn plants, 79-83
- Corn—
  - iron absorption by, 79, 85.
  - lime on peat soil for, 81.
  - potash on peat soil for, 80.
- Cottonseed meal, effect on carbon dioxide production in soils, 5, 6.
- Culture solution, effect of hydrogen-ion concentration on absorption of nitrate and ammonium by tomato plants, 203
- Cyanamid, *see* Calcium cyanamid.
- Electrodialysis, study of the nature of soil phosphates by, 253.
- Fertilizer—*see also* Nitrogen, Phosphorus, Potassium, Urea.
  - calcium cyanamid as a, 305
  - effect on—
    - cellulose decomposition, 12.
    - pineapple yield, 90.
- Fungi, number of, in soils variously treated, 11; *see also* Microorganisms.

- Hydrogen-ion concentration—  
as affected by—  
rainfall, 86.  
sulfur, 3.  
effect on—  
absorption of nitrate and ammonium  
by peach trees from culture solu-  
tion, 357.  
absorption of nitrate and ammonium  
by tomato plants from culture  
solution, 203, 459.  
cellulose decomposition, 12.  
corn in Hawaiian soils, 91.  
iron solubility in soil, 88.  
organic matter decomposition, 4.  
electrometric titration method of finding  
the, of soils, 49.  
quinhydrone electrode in determining the,  
of soils, 55.
- Iron—  
absorption of, by corn plants, 79.  
acid-oxalate method of Tamm for re-  
moval of iron from soils, 140.  
availability of, to pineapple and corn, 85.  
effect on—  
corn, 91.  
phosphate fixation, 344.  
movement of, in podzol soils, method of  
study, 140.  
silicates, base exchange capacity of, 423.  
solubility in soil at different hydrogen-ion  
concentration values, 88.  
Kainit, effect on replaceable bases, 167.  
Laterites, phosphate fixation in, 344, 350.
- Legumes—  
cross inoculation of, 126.  
nodule formation on, as affected by nitro-  
gen additions, 431-443.  
Rhizobium species in relation to nodule  
formation in Florida, 117, 229.
- Lime—  
effect on—  
base exchange in soils, 335.  
building up soils, 335.  
carbon dioxide production in soils, 7.  
cellulose decomposition, 12.  
hydrogen-ion concentration in soils, 335.  
iron content of corn, 81.  
microorganisms in soils, 11.  
nitrogen content in soils, 335.  
organic matter in soils, 335.  
phosphorus content in soils, 335.  
potassium fixation in soils, 277.  
requirement, electrometric titration  
method for, 49.
- Lysimeters, magnesium studies with, 289.
- Magnesium, absorbed and exchangeable, 289.
- Manure, effect on carbon dioxide production  
in soils, 5, 6.
- Microbiology, tropical soil, studies, 109-115.
- Microorganisms—*see also* Bacteria, Fungi.  
as affected by—  
drying, 11.  
liming, 11.  
number of, in variously treated soils, 11.
- Moisture—  
equivalent, relation to settling volume, 21.  
relationships in a number of soils, 24.
- Muscovite, theories regarding the formation  
of, in soil, 284.
- Nitrate—  
effect on nodule formation in legumes, 437.  
nitrogen—  
for plants, 203, 357, 459.  
in plants, method of determining, 461.
- Nitrates—  
as affected by sulfur, 3  
effect on—  
alfalfa and vetch, 434.  
nodule formation in legumes, 437.  
reducing sugars in soybeans, 436.
- Nitrification, capacity for, in various  
treated soils, 11.
- Nitrogen—  
amido, in plants, method of determining,  
462.  
amino, in plants, method of determining,  
462.  
as affected by—  
lime, 335.  
sulfur, 3.  
carriers, comparison of, as a fertilizer, 309.  
effect on—  
carbohydrate content of roots, 433.  
carbon dioxide production in soils, 5.  
nodule formation on legumes, 431.  
pineapples, 90.  
Rhizobium, 387.
- forms of—  
for plants, 203, 357.  
in plant tissue, method of determining,  
462.  
humin, in plants, method of determining,  
462.  
soluble, in plants, method of determining,  
462.

Odén, Sven, an obituary note, 429.

**Organic—**

carbon, sulfur effect on, 3.

materials, effect of biological transformation of, on colloid dispersion, 179.

**matter—**

content of a number of soils, 22.

decomposition of, as affected by soil acidity, 1-14.

method of determining, Degtjareff method, 29.

value of, in building up soils, 335.

water-soluble, as affected by sulfur, 3.

Oxidation-reduction potentials in soils, 65-76.

Oxygen consumption by Rhizobium, 387.

Peaches, absorption of nitrate and ammonium by, from sand cultures, 357.

**Peat—**

American, Deposits (book review), 77

corn yield on, with potash, 80.

**Phosphates—**

fixation and penetration of, in soils, 343-355.

iron and aluminum, electrodialysis of, 257.

rock, versus acid phosphate, 483.

soil, electrodialysis as a means of studying, 253-266.

**Phosphorus—**

as affected by lime, 335.

**available—**

as affected by sulfur, 3.

difficultly, method of determining, 477.

capacity of a soil to fix, in difficultly available form, 477-482.

**effect on—**

availability of iron to pineapples and corn, 85.

corn in Hawaiian soils, 91.

relation of rainfall to, in soil, 87.

Pineapples, availability of iron to, as influenced by sulfur and phosphorus, 85.

**Plants—**

feeding of, by briquette fertilization, 445-457.

nitrogen forms in, methods of determining, 460.

Podzols, studies on, 137.

**Potash—**

effect on replaceable potassium, 99.

fixation of, in soils, 267-287.

muriate on peat soil for corn, 80.

salts, effect on replaceable bases, 167.

**Potassium—**

**effect on—**

pineapples, 90.

soil acidity, 176.

exchangeable, in soils, 39.

nitrate, effect on nodule formation in legumes, 439.

replaceable, relation to—

rainfall, 87.

response to potash in Hawaiian soils, 99.

Protein kinship of *Crotalaria* species, 233.

**Rhizobium—**

physiological studies on, 387-401.

relation to nodule formation on legumes, 117-135, 227.

serological properties of, 230.

Silica-sesquioxide ratio, relation to settling volume of colloids, 23.

Silicates, ferric, base exchange capacity of, 423.

Sodium, exchangeable, in soils, 39.

**Soil—**

**acidity—**

as affected by potassium fertilizers, 176.

effect on organic matter decomposition, 1-14.

carbon dioxide, *see* Carbon dioxide

colloidal behavior, laws of, 403-427.

colloids—*see also* Colloids.

relation of settling volume of, to silica-sesquioxide ratio, 23

settling volume of, 22.

Colts Neck, analysis of, 484.

**horizons—**

mechanical composition of, 19, 20.

organic matter content in various, 22.

settling volume of, 18.

lateritic (Waipio), fixation of phosphorus by, 480.

Memphis silt loam, fixation of phosphorus by, 480.

Miami silt loam, fixation of phosphorus by, 480.

microbiology, *see* Microbiology.

minerals, potash fixation by, 277.

moisture equivalent, *see* Moisture equivalent.

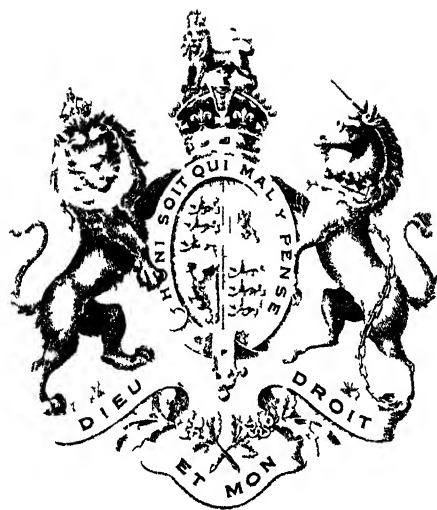
muscovite, theories regarding the formation of, in, 284.

organic matter, *see* Organic matter.

phosphates, *see* Phosphates.

shrinkage, an apparatus for measuring, 59-63.





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